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(54) Title: BIOPOLYMER THICKENER

(57) Abstract: A novel strain of *Lactococcus lactis* subspecies *cremoris* ("Ropy 352") has been identified and isolated. Ropy 352 produces a previously unknown exopolysaccharide (EPS 352) that when expressed in or added to milk, imparts highly desirable sensory characteristics to the milk, including making the milk very thick, with a very smooth mouth-feel, and slightly sweet with an obvious "chewable-bite".



WO 01/57234 A2

- 1 -

BIOPOLYMER THICKENER**ACKNOWLEDGMENT OF GOVERNMENT SUPPORT**

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5 National Dairy Promotion and Research Board (i.e. Dairy Management Inc., DMI)
and USDA/CSREES Special Research Grant. Accordingly the government has
certain rights in this invention.

FIELD OF INVENTION

10 The field of the invention relates to biopolymers, enzymes that are contained
within biopolymer synthesis pathways, nucleic acid sequences encoding such
enzymes, and to organisms that make such biopolymers, wherein such biopolymers
may be used to thicken liquids including liquid foods, as well as an additive to
pharmaceuticals, beauty products, and coating agents.

15

BACKGROUND

Microbial polysaccharides are used for a broad variety of industrial
applications including food production, chemical production (e.g., detergents,
cosmetics, paints, pesticides, fertilizers, flocculants, film formers, lubricants and
20 explosives), pharmaceutical production and waste treatment. In food production,
microbial polysaccharides are commonly used as thickening, gelling and
homogenizing agents. When added to a liquid, microbial biopolymers contribute to
viscosity, emulsion stabilization, surface tension and adhesiveness. Thickening
applications are particularly important in the production of solid and semi-solid food
25 products including dairy and non-dairy foods such as yogurt, buttermilk, salad
dressings, cheese, and ice-cream. Thickening of liquid foods is desirable because of
consumer preference for such thickened foods, which have a characteristic texture
and "mouth feel." Thickening of liquid drinks is also desirable for use with elderly
people who frequently have problems swallowing low-viscosity liquids (e.g., milk
30 and fruit juices) due to an impaired swallowing reflex. The addition of thickener to
such drinks facilitates swallowing and reduces aspiration of liquid into the trachea.

- 2 -

Currently the only microbial polysaccharides used to any appreciable extent in industry are dextran, produced by *Leuconostoc mesenteroides*, xanthan gum, produced by *Xanthomonas campestris*, and gellan gum, produced by *Aureomonas elodea* ATCC31461 (Crescenzi, *Biotech. Prog.* 11:251-259, 1995). Xanthan gum was approved by the U.S. Food and Drug Administration (FDA) for use in foods in 1969. Today it is used in many foods such as bakery fillings, canned foods, frozen foods, pourable dressings, sauces, gravies, processed cheeses, and juice drinks. Xanthan gum is also used in oil recovery, pharmaceuticals, beauty products, and coating agents.

Unfortunately, *Xanthomonas campestris* is a less than ideal source of polysaccharides for use in food production, since it is known to be pathogenic, and the biopolymer it produces has long been suspected of being pyrogenic (fever-inducing). Although xanthan gum is classified as "Generally Regarded as Safe" (GRAS) by the Food and Drug Administration (FDA), *Xanthomonas campestris* is not.

Lactic acid bacteria (LAB) are classified GRAS, and have been used for centuries in fermented dairy products such as yogurt, cheese, and sour-cream. A characteristic of some LAB in food production processes is their production of exopolysaccharides (EPS). EPS provide improved viscosity and mouth-feel while also preventing syneresis (separation) in fermented food products. Despite their ability to produce EPS, LAB are not generally used as sources of thickening agents (either within a milk-based culture or as a source of exogenous EPS) because the EPS-positive phenotype is readily lost (Dierkesen et al., *J. Dairy Sci.* 80(8):1528-1536, 1997). The LAB strain described in this disclosure stably produces EPS when cultivated on appropriate media.

SUMMARY OF THE DISCLOSURE

A natural isolate of *Lactococcus lactis*, named "*Lactococcus lactis* subspecies *cremoris* Ropy 352," hereinafter referred to simply as "Ropy 352", has been isolated. This strain contains a plasmid (EPS plasmid) that encodes at least 13 active genes (Figure 3). The enzymes encoded by these genes allow the bacteria to produce a previously unknown exopolysaccharide ("EPS 352"). Hence, in addition

- 3 -

to providing EPS 352, the present invention also provides the nucleic acid sequences and the corresponding amino acid sequences of 13 of the open reading frames (ORFs; SEQ ID NO: 10) found on the EPS 352 plasmid.

5 EPS 352, when expressed in or added to milk or other liquids, imparts desirable sensory characteristics to the milk, including making the milk very thick, with a very smooth mouth-feel, and slightly sweet with an obvious "chewable-bite." Ropy 352 producing EPS, or EPS 352 alone may be added to any milk-based or non milk-based product, including any liquid food product, to produce these sensory characteristics. In the Ropy 352 strain, the biosynthesis of EPS 352 is controlled by
10 genes carried outside the chromosome on a plasmid of about 32 kb ("EPS 352 plasmid"). Precedent predicts that the EPS 352 genes are linked in an operon like fashion. The EPS 352 plasmid has been isolated from the Ropy 352 organism, and the plasmid has been transformed into a plasmid free nonropy laboratory strain of *Lactococcus*, MG1363. (Gasson, *J. Bacteriol.* **154**:1-9, 1983.) The plasmid encoded
15 EPS 352 genes are expressed in the transformed strain, producing a ropy EPS, which imparts desirable sensory characteristics (as detailed below) to milk-based media.

One aspect of the invention provides the isolated *Lactococcus lactis* subspecies *cremoris* Ropy 352 organism (Ropy 352) as deposited under the rules of the Budapest Treaty, USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229.
20 Ropy 352 can be added to liquids (e.g., solids, semi-solids and gels) to cause thickening. Such thickening is desirable for use in creating products such as food products, beauty care products, and pharmaceuticals. Additionally, the Ropy 352 organism can be used to produce food products by fermentation of a food substrate with a culture of the Ropy 352 organism. Accordingly, the invention also provides
25 the products made through the addition of the Ropy 352 culture.

Another aspect of the invention provides the purified exopolysaccharide EPS 352. EPS 352 can be added to liquids to produce food products as well as other products such as pharmaceuticals. Examples of such liquids include, liquid food substrates, such as milk-based liquids, soy-based liquids, fruit juice, and whey-based
30 liquids. Accordingly the invention also provides the products made through the addition of EPS 352.

- 4 -

Yet another aspect of the invention provides the plasmid (contained in the deposited bacterial strain NRRL B-30229) that contains the open reading frames that encode the enzymes necessary for the production of EPS 352. This plasmid is approximately 32 kb in size. The identification of the plasmid allows for the
5 production of EPS 352 by transgenic organisms that have been transformed with the EPS 352 plasmid. Furthermore, these transgenic organisms can be added to liquids to generate food products.

Another aspect of the invention provides methods of using the individual enzymes encoded by the EPS 352 plasmid for the production of modified
10 exopolysaccharides. Used in these methods the enzymes derived from the nucleic acid sequence of the EPS 352 plasmid can be combined with other genes that code for exopolysaccharide biosynthetic pathways enzymes such that the exopolysaccharide produced is distinct from that of the disclosed EPS 352. Furthermore, these methods can be practiced *in vitro* or *in vivo*. (Stingele et al.,
15 *Mol. Microbiol.* **32**(6):1287-1295, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6453, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; and Klerrebezem et al., *Antonie van Leeuwenhoek* **76**:357-365, 1999).

Another aspect of the invention provides methods of using EPS 352 in various pharmaceutical formulations. Used in this context EPS 352 can be
20 incorporated dry into pill formulations or into liquids to increase the viscosity of the formulation and facilitate delivery of the active ingredients.

Another aspect of the invention provides methods of using EPS 352 in various beauty products, such as hair shampoos, hair bleaching compositions, hair conditioners, hair gels and mousse, skin creams, nail varnishes, facial foundation,
25 skin tanning gels, hair removers, shaving creams and in pill coatings, children's products (i.e., crayons, non-toxic glues), in addition to various industrial processes. (Hilger et al., *J. Environ. Eng.* **125**(12):1113, 1999 and Shah et al., *Appl. Biochem. Biotech.* **82**(2):81, 1999.)

30 SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three-

- 5 -

letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood to be included by any reference to the displayed strand.

5 SEQ ID NO: 1 shows the nucleic acid sequence of a portion of the EPS 352 plasmid.

 SEQ ID NO: 2 shows the amino acid sequence of the enzyme designated "R" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 3 shows the amino acid sequence of the enzyme designated "X" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

10 SEQ ID NO: 4 shows the amino acid sequence of the enzyme designated "A" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 5 shows the amino acid sequence of the enzyme designated "B" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

15 SEQ ID NO: 6 shows the amino acid sequence of the enzyme designated "C" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 7 shows the amino acid sequence of the enzyme designated "D" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 8 shows the amino acid sequence of the enzyme designated "E" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

20 SEQ ID NO: 9 shows the amino acid sequence of the enzyme designated "O" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 10 shows the amino acid sequence of the enzyme designated "P" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

25 SEQ ID NO: 11 shows the amino acid sequence of the enzyme designated "F" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 1.

 SEQ ID NO: 12 shows the nucleic acid sequence encoding Eps "M" and Eps "N."

30 SEQ ID NO: 13 shows the amino acid sequence of the enzyme designated "N" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 12.

- 6 -

SEQ ID NO: 14 shows the amino acid sequence of the enzyme designated "M" in Figure 4, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 12.

5 SEQ ID NO: 15 shows the nucleic acid sequence encoding the enzyme designated "U."

SEQ ID NO: 16 shows the amino acid sequence of Eps "U," which is encoded by SEQ ID NO: 15.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figure 1** describes the degree of phosphate protonation. As sodium hydroxide is added to the polysaccharide solution, there is only one inflection in the titration profiles, indicating that the phosphate group in the EPS 352 is in the form of a phosphodiester linkage rather than as the monoester, which would have shown 2 inflection points.

15 **Figure 2** shows double stranded sequence data from the EPS 352 plasmid and the corresponding amino acid sequences named EpsM and EpsN. The insertion site of the ISS1 element is indicated in EpsN and which confers a non-ropy phenotype in Ropy 352, thus linking these two open reading frames to EPS 352 expression.

20 **Figure 3** shows the alignments of the ORF designated "N" in Figure 4 and the ORF designated "M" in Figure 4 to each other as well as to an enzyme (EpsG) involved in eps biosynthesis in *Lactococcus lactis* NIZOB40. The overall identity between ORF "M" and EpsG is 24% and between ORF "N" and EpsG is 25%.

25 **Figure 4** is a diagram of the organization of the genes on the EPS 352 plasmid. The large arrows with letters inside represent genes and their orientation. The square with the letter X is a non-functional gene as it is missing its beginning (5' prime sequence). Eps ORFs are designated M, N, O, and P. The site of the ISS1 insertion, which disrupted EPS 352 production, is indicated by an downward pointing arrow that points to a position in Eps N.

30 **Figure 5** shows the DNA and amino acid sequence of the entire EPS operon from upstream of the promoter to downstream of the terminator. This sequence is

- 7 -

6850 bp in length. The starts of the open reading frames are labeled with the gene name (corresponding to Figure 4) printed in the right margin.

Figure 6 shows the nucleic acid sequence of Eps U. The start and stop codons are underlined.

5

DETAILED DESCRIPTION

DEFINITIONS and ABBREVIATIONS

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes VII*, Oxford University Press, 1999 (ISBN 0-19-879276-X);
 10 Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology* Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

W/V means weight per unit volume.

15

kDa means kilodaltons.

MWCO means molecular weight cutoff

TCA means trichloroacetic acid.

Mol % means molar percent

mPA-s means millipascals

20

n.d. means none detected.

***Lactococcus lactis* subspecies *cremoris* Ropy 352 ("Ropy 352")** is the organism deposited under the Budapest Treaty as USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229. Ropy 352 has the characteristic property of producing the exopolysaccharide EPS 352 under suitable growth conditions, e.g.,
 25 streaked onto whey agar or defined lactococcal medium containing glucose agar plates and incubated at 30°C.

EPS 352 is an exopolysaccharide that is produced by Ropy 352 and that has the following characteristics:

30	Composition: Glucose: range of 54% to 58% Galactose: range of 42% to 46%
	Charged: Yes
	Molecular weight: range of 800,000 to 8,000,000

- 8 -

(average of 1,600,000)

Phosphorous: Present in backbone or sidechain

Structure: Endpoints: galactose; Branchpoints: glucose

5 Several gene products are required for EPS 352 biosynthesis. The EPS biosynthetic genes are located extrachromasomally on the EPS 352 plasmid. Precedent indicates that these genes are organized in an operon like fashion.

EPS 352 plasmid is an extrachromosomal plasmid of approximately 32 kb in size that carries the EPS 352 biosynthetic genes. Current methods used to
10 estimate plasmid size are not exact. For instance, the perceived size of a plasmid may be effected by the degree of relaxation of the plasmid and the degree to which proteins may be associated with the plasmid. Thus, the EPS 352 plasmid is believed to be about 32 kb in size, and may be, for example, from 30 to 38 kb in size. Several research groups have linked EPS biosynthesis with plasmids of various sizes: 6.8 kb,
15 25.8 kb, 28 kb, 40.2 kb, and 45.5 kb (Vescovo et al., *Biotech. Letters II* **10**:709-712, 1989; Neve et al., *Biochimie* **70**:437-442, 1988; Vedamuthu et al., *Appl. Environ. Microbiol.* **51**:677-682, 1986; Kranenburg et al. *Mol. Microbiol.* **24**:387-397, 1997; and Von Wright et al., *Appl. Environ. Microbiol.* **53**:1385-1386, 1987).

Food means any eatable or drinkable substance consumed by humans or
20 animals, e.g., milk, cream, dairy products, soy products, fruit juice, vegetable juices, ice cream, soups, etc.

Food Product means any food that is produced by altering its original state, e.g., milk to which has been added EPS 352.

Milk is used broadly herein to include all dairy products regardless of fat
25 content or lactose content. The term as used herein also includes substances commonly used in place of milk, such as soy used as "soy milk". The term also includes milk products from animals other than cows, including goat milk.

Liquid as used herein includes fluids with varying degrees of fluidity including highly fluid liquids such as non-fat milk, thicker liquids such as full fat
30 milk and cream, semi-solid substances, and gels such as yogurt and other fermented milk products. A liquid may be altered from its original state to produce an altered liquid, e.g., an adhesive solution, a paint emulsion, a lubricant, or a fruit juice to which EPS 352 has been added.

- 9 -

A Milk-Based liquid is any liquid wherein milk forms an appreciable percentage of the total volume of the liquid. For example, a liquid having 0.10% or more of milk solids.

A Soy-Based liquid is any liquid wherein soy forms an appreciable
5 percentage of the total volume of the liquid. For example, a liquid having 0.10% or more of soy solids

To Thicken means to decrease fluidity and **increase viscosity**.

Thickener means any substance used to thicken, including, for instance, exopolysaccharides. A thickener may be produced by organisms cultured within a
10 medium or may be added exogenously to a medium.

Mouth-feel is a term of art used in the food industry to describe sensory characteristics of a food. It has the same meaning as the word "texture" which has been previously defined as "the composite of the structural elements of the food and the manner in which it registers with the physiological sense" (Szczesniak, *J. Food Science* 28:385-389, 1963), or "the composite of those properties which arise from
15 the physical structural elements and the manner in which it registers with the physiological senses" (Sherman, *J. Food Science* 27:381-385, 1970).

Pharmaceutical a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

Beauty care product is an externally applied product that is intended to alter
20 the appearance of the subject to which it has been applied.

Coating agent an agent applied to the exterior surface of an object. A coating agent generally forms a thin layer on the surface of the object.

Transformed refers to a cell into which a nucleic acid molecule has been
25 introduced by molecular biology techniques. The term encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transformation with plasmid vectors, transfection with viral vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Purified does not require absolute purity; rather, it is intended as a relative
30 term. Thus, for example, a purified polysaccharide preparation is one in which the subject polysaccharide is more pure than in its natural environment within a cell or within a cell culture medium. Generally, a polysaccharide preparation is purified

- 10 -

such that the polysaccharide represents at least 50% of the total polysaccharide content of the preparation.

Isolated an *isolated* nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acids.

ORF is an open reading frame. An ORF is a contiguous series of nucleotide triplets coding for amino acids. These sequences are usually translatable into a peptide.

Operably linked means a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

Probe is an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

Target Nucleic Acid is a nucleic acid that hybridizes with a probe. The conditions under which hybridization occurs may vary with the size and sequence of the probe and the target sequence.

By way of illustration, only a hybridization experiment may be performed by hybridization of a DNA probe (for example, a probe derived from the EPS 352 plasmid labeled with a chemiluminescent agent) to a target DNA molecule which has been electrophoresed in an agarose gel and transferred to a nitrocellulose membrane by Southern blotting (a technique well known in the art and described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., vols. 1-3, Cold Spring Harbor, New York, 1989).

- 11 -

Hybridization with a radio-labeled probe is generally carried out in a solution of high ionic strength such as 6 x SSC at a temperature that is 20°C-25°C below the melting temperature, T_m , described below. For such Southern hybridization experiments where the target DNA molecule on the Southern blot contains 10 ng of DNA or more, hybridization is typically carried out for 6-8 hours using 1-2 ng/mL radiolabeled probe. Following hybridization, the nitrocellulose filter is washed to remove background hybridization. The wash conditions should be as stringent as possible to remove background hybridization but to retain a specific hybridization signal. The term T_m represents the temperature above which, under the prevailing ionic conditions, the radiolabeled probe molecule will not hybridize to its target DNA molecule. The T_m of such a hybrid molecule may be estimated from the following equation:

$$T_m = 81.5^\circ\text{C} - 16.6 (\log_{10} [\text{Na}^+]) + 0.41 (\% \text{G+C}) - 0.63 (\% \text{ formamide}) - (600 / l)$$

Where l = the length of the hybrid in base pairs. This equation is valid for concentrations of Na^+ in the range of 0.01M to 0.4M, and it is less accurate for calculations of T_m in solutions of higher $[\text{Na}^+]$. The equation is primarily valid for DNAs whose G+C content is in the range of 30% to 75%, and applies to hybrids greater than 100 nucleotides in length (the behavior of oligonucleotide probes is described in detail in Ch. 11 of Sambrook et al., 1989).

Generally hybridization wash conditions are classified into categories, for example very high stringency, high stringency, and low stringency. The conditions corresponding to these categories are provided below.

Very High Stringency (detects sequences that share 90% sequence identity)

25	Hybridization in	5x	SSC	at	65°C	16 hours
	Wash twice in	2x	SSC	at	Room temp.	15 minutes each
	Wash twice in	0.2x	SSC	at	65°C	20 minutes each

- 12 -

High Stringency (detects sequences that share 80% sequence identity or greater)

5 Hybridization in 3x SSC at 65°C 16 hours
 Wash twice in 2x SSC at Room temp. 15 minutes each
 Wash twice in 0.5x SSC at 55°C 20 minutes each

Low Stringency (detects sequences that share greater than 50% sequence identity)

10 Hybridization in 3x SSC at 65°C 16 hours
 Wash twice in 2x SSC at Room temp. 20 minutes

The above example is given entirely by way of theoretical illustration. One skilled in the art will appreciate that other hybridization techniques may be utilized and that variations in experimental conditions will necessitate alternative calculations for stringency.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids that may be substituted for an original amino acid in a protein and that are regarded as conservative substitutions.

TABLE 1

Original Residue	Conservative Substitutions
ala	ser
arg	lys
asn	gln; his
asp	glu
cys	ser
gln	asn
glu	asp
gly	pro

- 13 -

Original Residue	Conservative Substitutions
his	asn; gln
ile	leu; val
leu	ile; val
lys	arg; gln; glu
met	leu; ile
phe	met; leu; tyr
ser	thr
thr	ser
trp	tyr
tyr	trp; phe
val	ile; leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative. For instance, changes in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Primers are short nucleic acids, preferably DNA oligonucleotides 10 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

- 14 -

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30, 40, 50, 60, 70, 80, 90, 100, or 150 consecutive
5 nucleotides of the disclosed nucleic acid sequences.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor, New York, 1989; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publ. Assoc. & Wiley-Intersciences, 1987;
10 Innis et al., *PCR Protocols, A Guide to Methods and Applications*, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as *Primer* (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

Recombinant nucleic acid is a sequence that is not naturally occurring or
15 has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook et al. (1989). The term recombinant includes nucleic acids
20 that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector, used to transform a cell.

Sequence identity: The similarity between two nucleic acid sequences or
25 between two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences.

Methods for aligning sequences for comparison are well known in the art.
30 Various programs and alignment algorithms are described in: Smith & Waterman, *Adv. Appl. Math.* **2**:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* **48**:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* **85**:2444, 1988; Higgins & Sharp,

- 15 -

Gene **73**:237-244, 1988; Higgins & Sharp, *CABIOS* **5**:151-153, 1989; Corpet et al., *Nucleic Acids Research* **16**:10881-10890, 1988; Huang, et al., *CABIOS* **8**:155-165, 1992; and Pearson et al., *Methods in Molecular Biology* **24**:307-331, 1994. Altschul et al., *J. Mol. Biol.* **215**:403-410, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST™) (Altschul et al., *J. Mol. Biol.* **215**:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. BLAST™ can be accessed on the internet at NCBI website. A description of how to determine sequence identity using this program is available at the web site. As used herein, sequence identity is commonly determined with the BLAST™ software set to default parameters. For instance, blastn (version 2.0) software may be used to determine sequence identity between two nucleic acid sequences using default parameters (expect = 10, matrix = BLOSUM62, filter = DUST (Tatusov and Lipmann, in preparation as of December 1, 1999; and Hancock and Armstrong, *Comput. Appl. Biosci.* **10**:67-70, 1994), gap existence cost = 11, per residue gap cost = 1, and lambda ratio = 0.85). For comparison of two polypeptides, blastp (version 2.0) software may be used with default parameters (expect 10, filter = SEG (Wootton and Federhen, *Computers in Chemistry* **17**:149-163, 1993), matrix = BLOSUM62, gap existence cost = 11, per residue gap cost = 1, lambda = 0.85).

For comparisons of amino acid sequences of greater than about 30 amino acids, the "Blast 2 sequences" function of the BLAST™ program is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 45%, at least 50%, at least 60%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity.

- 16 -

METHODS

General Methods

The present invention utilizes standard laboratory practices for the cloning, manipulation and sequencing of nucleic acids, purification and analysis of proteins and other molecular biological and biochemical techniques, unless otherwise stipulated. Such techniques are explained in detail in standard laboratory manuals such as Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor, New York, 1989; and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publ. Assoc. & Wiley-Intersciences, 1989. Other techniques specific to *Lactococcus* are discussed in the inventors' publications including: Dierksen et al., *Genetics of Streptococci, Enterococci and Lactococci*, (Ferretti et al., eds.), 1995; Basel, *Dev. Biol. Stand* **85**:469-480, 1995; Dierksen et al., *J. Dairy Sci.*, **80**(8):1528-1536, 1997; and Knoshaug et al., *J. Dairy Sci.* **83**:633-640, 2000.

1. Growth and Characterization of the Ropy 352 organism.

The EPS 352 producing organism, *Lactococcus lactis* subspecies *cremoris* Ropy 352, was isolated, classified and deposited under the Budapest Convention as USDA-ARS-NCAUR-NRRL deposit number NRRL B-30229. Ropy 352 may be obtained on demand from the USDA-ARS-NCAUR-NRRL at Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research (NCAUR), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), 1815 North University Street, Peoria, IL 61604 U.S.A. Ropy 352 was streaked onto whey agar or defined lactococcal media containing glucose (DLMG) agar. Whey agar (Vedamuthu et al., *Appl. Microbiol.* **51**:677-682, 1986) made as previously described with the following modifications: yeast extract (5 g, Difco Laboratories, Detroit, MI) and sodium β -glycerophosphate (19 g, Sigma Chemical Co., St. Louis, MO) were added to the centrifuged supernatant and the volume brought up to 600 mL. The second part of the media consisted of 15 g of agar and 3 drops of antifoam A (Sigma) in 400 mL of water. Both portions were autoclaved for 12 min; removed promptly, cooled to 50°C, mixed, and poured into sterile petri plates. DLMG agar (Molenaar et al., *J. Bacteriol.* **175**:5438-5444,

- 17 -

1993.) was prepared as two parts; part one consisted of the base media which was prepared in 758 mL of water, heated to dissolve the components, mixed with 10 mL of the metals, vitamins, and nucleic acid solutions and 12 mL of 20% glucose or lactose solution, filter sterilized, and heated to 55°C in a water bath. Part two
5 consisted of 10 g of agar and 2 drops of antifoam A (Sigma) which were mixed into 200 mL of water, autoclaved, and cooled to 55°C. Part one was mixed into part two and poured into sterile petri plates. Ropy 352 was streaked onto plates and incubated at 30°C to produce macroscopic, individual, EPS 352 producing colonies of Ropy 352 (procedure described in inventors' publications listed above).

10 The EPS 352 may be recognized by the formation of viscous ropes greater than five mm in length originating from a whey agar or DLMG agar. Whey agar plates were incubated at 30°C for 48 h. Characteristic ropy phenotype is apparent from viscous rope greater than 5 mm formed when a colony is touched with a sterile toothpick. These ropes became visible when the colony was touched with a sterile
15 toothpick and the toothpick was drawn away from the colony, thus, stretching the EPS 352 out. An additional way to recognize EPS 352 is by the formation of viscous ropes in liquid milk inoculated with Ropy 352 organism. Liquid milk was sterilized by steaming for 30 min and 10 mL of milk were inoculated with 0.5 mL of an overnight Ropy 352 culture. The milk was incubated for 18 hours at 30°C and
20 visually examined for ropy EPS expression. These viscous ropes were visualized by touching the milk with a toothpick and drawing the toothpick away from the milk.

2. Purification and Characterization of EPS 352.

An individual EPS 352 producing Ropy 352 colony from a whey agar plate
25 was picked and used to inoculate 1 L of polysaccharide production medium in a 2.8 L Fernbach flask. The medium was cultured at 30°C for 16 to 20 hours without shaking. The polysaccharide production medium consisted of 10% w/v nonfat milk in water, which was prepared by stirring 100 g dry milk powder into 1 L deionized water at room temperature for 1 hour and then sterilizing the mixture in an autoclave
30 for 12 minutes at 120°C.

Ropy 352 culture broths were transferred to 500 mL centrifuge bottles and insoluble fractions were pelleted at 10 K x g for 20 minutes. Clarified supernatants

- 18 -

were dialyzed (6-8 kDa MWCO, Spectra/Por 1; Spectrum Laboratories, Inc., Laguna Hills, CA) against water containing 0.02% sodium azide for at least 24 hours.

An equal volume of absolute ethanol was added to the contents of the dialysis tubing and stirred in an ice bath. Ropy 352 cultures formed a precipitate of elongated ropes that were collected by centrifugation as described above. This was termed the Ropy fraction and contained EPS 352.

From 1 L of 10% nonfat milk medium, 34 mg of total polysaccharide was recovered from Ropy 352 cultures after centrifugation and dialysis. The polysaccharide responsible for the ropy characteristic (EPS 352) was purified by precipitation with 50% ethanol, followed by trichloroacetic acid (TCA) removal of residual protein. This Ropy fraction contained 10 mg of polysaccharide and was essentially protein free (<20 µg/mg in the final product). The Ropy fraction also contained 2.3 µg phosphorus/mg polysaccharide.

Compositional analysis of EPS 352 revealed a repeating structure composed of approximately 54% to 58% glucose, and 42% to 46% galactose. Compositional data suggests a novel structure for EPS 352 with glucose as the branch residue and galactose located at the end points.

The predominant sugar found in EPS 352, at 36 mol%, is (1,4)-linked glucose. The only sugar found as terminal non-reducing end groups (i.e., had a single linkage position) was galactose at 27 mol%; this quantity is indicative of a highly branched structure. A (1,4,6)-linked glucose residue was found at a concentration of 21 mol%; the three linkage sites indicate that it is a branch point in this structure. The least represented sugar was the (1,4)-linked galactose, which occurred at a concentration of 15 mol%. Results from this analysis are listed in

Table 2:

Table 2
Identification of permethylated PAAN (Peracetylated aldononitrile)
derivatives from Ropy 352 and Ropy polysaccharides

PAAN methyl sugar	Linkage site	Ropy fraction from Ropy 352 (mol%)
2,3,4,6-tetra- <i>O</i> -methyl galactose	1	27
2,3,6-tri- <i>O</i> -methyl galactose	1,4	15
2,4,6-tri- <i>O</i> -methyl galactose	1,6	n.d. (none detected)
2,3,4-tri- <i>O</i> -methyl galactose	1,6	n.d.
2,3,6-tri- <i>O</i> -methyl glucose	1,4	36
2,3,4-tri- <i>O</i> -methyl glucose	1,6	n.d.
3,4,6-tri- <i>O</i> -methyl mannose	1,2	n.d.
2,3-di- <i>O</i> -methyl glucose	1,4,6	21
3,4-di- <i>O</i> -methyl glucose	1,2,6	n.d.
2,4-di- <i>O</i> -methyl mannose	1,3,6	n.d.

The degree of phosphate protonation is shown in Figure 1. As sodium hydroxide was added to the polysaccharide solution, there was only one inflection in the titration profiles, indicating that the phosphate group in the Ropy fraction polysaccharides is in the form of a phosphodiester linkage rather than as the monoester, which would have shown 2 inflection points.

3. Viscosity of Milk Culture During 25 hour Fermentation with Ropy 352.

1 L of milk was inoculated with a single whey agar-grown colony of Ropy 352. Viscosity was measured with a Brookfield model LVTDV-I digital viscometer (Stoughton, MA) using a LV1 spindle.

The viscosity of the Ropy 352 culture reached a value of 44000 mPA-s at 24 hours, compared to an initial viscosity of 1 mPa-s (see Table 3). This data verifies the phenotypic observation that Ropy 352 culture thickens a liquid food product (milk).

Table 3
Viscosity change (in mPa-s) after 24 h.

Strain	Sample	0 h	24 h
Ropy 352	Fermented milk	1.0	44000
No cells	Milk	1.0	1.0

- 20 -

4. Isolation and Characterization of the Biosynthetic EPS 352 Plasmid.

The EPS 352 plasmid is a plasmid of about 32 kb in size that may be isolated from Ropy 352. A 2.2 KB fragment from the EPS 352 plasmid (Figure 2) and a 6.85 kb fragment (Figure 4) have been sequenced. These sequences encode ORFs M and N which show homology to a class of sugar transfer enzymes (glycosyltransferases) known to be involved in EPS biosynthesis (Figure 2). Several restriction endonucleases cut this plasmid, including *EcoRI*, *EcoRV*, *HindIII*, *SacI*, *SphI*, *DraI*, *HincII*, *NdeI*, *Sau3AI*, and *SpeI*.

The EPS 352 plasmid contains all biosynthetic genes coding for the enzymes needed to make EPS 352. This was demonstrated by the following experiment. The EPS 352 plasmid, containing an erythromycin resistant encoded insertion element for selection, was isolated from a culture of Ropy 352 using DNA preparation methods as described in Knoshaug et al., *J. Dairy Science* **83**:633-640, 2000. (Ref for plasmid DNA isolation: O'Sullivan et al., *Appl Environ Microbiol.* **59**:2730-2733, 1993). This DNA was used to transform a plasmid-free nonropy lactococcal strain, MG1363 by electroporation as described (Dornan et al., *Lett. Appl. Microbiol.* **11**:62-64, 1990; Holo et al., *Appl. Environ. Microbiol.* **55**:3119-3123, 1989). Cells were grown for 24 hours in M17-glucose media supplemented with 0.3 M sucrose and 2% (MG1363) or 0.5% (Ropy352) glycine. Cells were pelleted, washed in cold 0.3 M sucrose three times, and resuspended in 200 µl of 0.3 cold M sucrose. DNA was added to the cells and the mixture was transferred to a chilled electroporation cuvette (0.2 cm gap). The cells were shocked (2.5 kV, 200 ohms, 25 µF) and resuspended in 8 mL of growth media supplemented with 0.3 M sucrose and 50 ng/mL em. Cells were allowed to recover for 1.5 hours before plating on whey agar containing 2 µg/mL em. Erythromycin resistant transformants were selected, and then screened for the ropy EPS 352 phenotype. MG1363 containing the EPS 352 plasmid was analyzed by Southern blot to verify the presence of the plasmid. The probe used was 1.6 kb long and specific to the Ropy 352 EPS ORF M and ORF N genes. Results demonstrated that the probe reacted with a 32 kb plasmid in Ropy352 (un-nicked and nicked forms) and with a 37 kb plasmid in EK356 (EPS

- 21 -

352 plasmid containing a 5.4 kb erythromycin resistant encoded insertion element for selection; un-nicked and nicked forms).

The southern blot analysis was additionally confirmed by testing the transformed bacteria for the Ropy phenotype. Results showed that the phenotypic
5 carried over to the MG1363 strain.

5. Production of Food Products by Adding EPS 352 to a Food Substrate.

EPS 352 can be added to a liquid food substrate to increase viscosity and thickness of the liquid and to enhance texture and mouth-feel. Liquid food
10 substrates may include, but are not limited to: milk (including low-fat and non-fat milk), milk-based liquids, whey-based liquids, soy-based liquids, fruit-juices, and oil-based liquids and emulsions. EPS 352 can be used to enhance the thickness and texture of, for example, yogurt, milk-shakes, fruit-juices, soy drinks, Scandinavian fermented milk products (e.g., "villi, "langfil," and "filmjolk,"), bakery fillings,
15 dressings, sauces and gravies. EPS 352 can also be added to solid or semi-solid food substrates to enhance the texture of, for instance, frozen foods, canned foods and cheeses. Thickness of the liquid food substrate will increase in proportion to the amount of EPS 352 added. EPS 352 may be added to any liquid food substrate in an amount necessary to produce the desired consistency. Determining an amount
20 necessary to produce a desired consistency is a simple matter of empirical experimentation.

A specific example of a food product made using EPS 352 is a thickened, non-fermented food product that has the qualities of yogurt, but without the need for fermentation. Milk (e.g., non-fat milk) can be used as a liquid food substrate to
25 which an amount of EPS 352 can be added, sufficient to cause thickening to a desired consistency. EPS 352 may be supplied in the form of an essentially pure powder and added directly to the milk. The powder may be mixed into the milk at room temperature using conventional methods and the mixture may then be aliquoted into sealed containers and pasteurized. Such a product would be low in
30 fat, have a yogurt-like consistency, and would not require fermentation, a step which is time-consuming, expensive and prone to microbial contamination.

- 22 -

6. Production of Milk-Derived Fermented Food Products by Adding a Pure Culture of the Ropy 352 Organism to a Food Substrate and Fermenting the Mixture.

Ropy 352 can be used to produce fermented food products such as yogurt
5 (and other products as listed above). Such products are described as probiotic (this refers to organisms who are ingested, such as the LAB, which contribute to the health and balance of the human's intestinal tract thus possibly protecting against disease and improving nutrition). During fermentation, Ropy 352 produces the EPS
10 352 exopolysaccharide which imparts desirable qualities to certain foods. In particular, EPS 352 gives fermented milk products a very smooth, rich mouth-feel with a slightly sweet flavor.

A specific example of a fermented food product made using Ropy 352 is yogurt. Milk (e.g., either whole, 2% or non-fat milk) can be used as a liquid food substrate to which a pure culture of Ropy 352 can be added. The culture may be
15 fermented, for instance at 30°C without shaking for 16 to 20 hours. The EPS 352 culture may be supplied in the form as an aliquot of liquid culture or an inoculum from an agar plate (such as milk or whey agar plate). Following fermentation, the fermented product may be aliquoted into sealed containers and pasteurized. A second specific example of a fermented food product made using Ropy 352 is a
20 power shake for the elderly and diet shakes for the obese. Trade names such as Slimfast™ or Ensure™ can be used as a liquid food substrate to which a pure culture of Ropy 352 can be added. Both Slimfast™ and Ensure™ were inoculated with a culture of Ropy352 and incubated at 30°C for 24 hours, respectively. The results showed that not only did Ropy 352 thicken these products, but it also added active
25 culture (probiotic) status.

The duration and temperature of fermentation may vary. Representative temperatures may range from about 17°C to 30°C and duration of fermentation of a batch culture may be from about 10 to 36 hours. Alternatively, fermentation may be done as a continuous culture with portions of the fermented product being
30 periodically removed.

7. The Use of Enzymes Derived from the EPS 352 Plasmid

Enzymes derived from the EPS 352 plasmid can be used either *in vitro* or *in vivo* to produce and or modify EPS structure. Furthermore, these enzymes can be modified through the inclusion of one or more conservative amino acid

- 5 substitutions, however, such conservative amino acid substituted variants will continue to maintain the same activity of the enzyme from which they are derived.

a. *in vitro*

Enzymes from the EPS 352 plasmid can be combined with other enzymes and substrates *in vivo*, such that an EPS is produced with the desired characteristics.

- 10 *In vitro* production of an EPS involves provide the isolated enzymes that are to be used in the synthesis as well as the various substrates necessary for the production of the EPS. Detailed examples of EPS production *in vitro* are well known in the art and can be found for example in Bossia et al., *Cell Mol Biol (Noisy-le-grand)* **42(5):737-58**, 1996 and

- 15 Semino et al., *J Gen Microbiol* **139** (Pt 11):2745-56, 1993.

b. *in vivo*

The enzymes produced from the expression of ORFs, such as ORF M (SEQ ID NO: 14), ORF N (SEQ ID NO: 13), ORF O (SEQ ID NO: 9), and ORF P (SEQ ID NO: 10) that are derived from the EPS 352 plasmid can be placed under the

- 20 control of heterologous control sequences. Such control sequences can be selected from constitutive promoters, inducible promoters, enhancers, and various terminators. Together the control sequence(s) operably linked to the ORF is termed the "transgene". The transgene can then be transformed into a host organism that supports the production of an EPS. Upon expression of the protein from the
- 25 transgene at least a portion of the EPS generated from the transformed host organism will be distinct from the non-transformed host organism.

It is also possible that the control sequences found in the EPS 352 plasmid can be used to express one of more of the ORF from the EPS 352 plasmid. Used in this way the "transgene" generated will be the result of using recombinant DNA

- 30 technology to manipulate the endogenous EPS 352 plasmid such that the naturally occurring EPS 352 plasmid is not intact. Such transgenes result from the introduction of additional copies of one or more of the ORFs that are in the naturally

- 24 -

occurring EPS 352 plasmid. It is also possible that enzymes from other EPS producing organisms will be introduced into the EPS 352 operon such that the host cell expresses an EPS that is distinct from the Ropy 352 disclosed herein.

5

EXAMPLES

1. Production of a Thickened Milk Product by Adding a Pure Culture of the Ropy 352 Organism to Milk and Fermenting the Mixture.

Ropy EPS 352 was expressed on plates containing whey agar and in liquid milk. The whey agar plates were incubated at 30°C for 48 hours. Colonies were then touched with a sterile toothpick to test for Ropy EPS 352 expression. Liquid milk was sterilized by steaming for 30 minutes. 10 mL of the sterilized milk were then inoculated with 0.5 mL of an overnight pure culture of the Ropy 352 organism. The milk was incubated for 18 hours at 30°C and visually examined for coagulation and ropy EPS 352 expression. Ropiness was indicated using a sterile glass rod to pull ropes from the milk.

2. Production of a Thickened Liquid Product by Adding a Pure Culture of the Ropy 352 Organism to Power Drinks Designed for the Elderly and Diet Drinks Designed for the Obese.

Ropy 352 was grown and EPS 352 was expressed in Slim Fast™ (Slim-Fast Foods Co., West Palm Beach, Florida) chocolate diet drink and Ensure™ (Abbott Laboratories, Abbott Park, Illinois) chocolate fortified drink. Slim Fast™ and Ensure™ drinks were inoculated with Ropy 352 and incubated for 18 hours at 30°C and visually examined for coagulation and ropy EPS 352 expression. Ropiness was determined using a sterile glass rod to pull ropes from the milk, and by visually examining how the fermented liquid poured from a flask.

3. Use of the EPS 352 Plasmid to Transform Cells and to Produce EPS 352.

The EPS 352 plasmid, containing an erythromycin resistant encoded insertion element for detection, was isolated from a culture of Ropy 352 using DNA preparation methods as described in Knoshaug et al., *J. Dairy Sci.* **83**:633-640, 2000 (and as referred to in the methods section of this document). This DNA was used to

- 25 -

transform a plasmid-free nonropy lactococcal strain, MG1363. Erythromycin resistant transformants were selected, and then screened for the ropy EPS 352 phenotype. Those displaying the ropy EPS 352 phenotype were Gram stained to verify that Gram positive cocci were present. MG1363 containing the EPS 352 plasmid was analyzed by Southern blot to verify the presence of EPS 352 plasmid. Presence of the EPS 352 plasmid in MG1363 correlated to the acquisition of the ropy EPS 352 phenotype.

4. Use of EPS 352 as a Substitute for Xanthan Gum

Xanthan gum is a high molecular weight polysaccharide derived from *Xanthomonas Campestris*. It contains D-glucose, D-mannose, and D-glucuronic acid as the dominant hexose units. For a more detailed discussion of the composition, physical and chemical properties, preparation, etc. of xanthan gum, see the following publications: Federal Register, Vol. 34, No. 53, Mar. 19, 1969, Subchapter B, Part 121, Subpart D; Keltrol, Technical Bulletin DB No. 18, Kelco Company, Clark, New Jersey.

Xanthan gum is currently used in a variety of compounds, as is evidenced by the fact that a search of the United States Patent and Trademark Office website on the Internet for "xanthan gum" in the claims of U.S. patents that have issued since 1976 identified 1,276 patents. These patents show xanthan gum being used in sprayable cleaning compositions (U.S. patent No. 5,948,743), hair conditioning shampoo (U.S. patent No. 5,948,739), ballpoint pen ink (U.S. patent No. 5,925,175), time-specific controlled release dosage formulations (U.S. patent No. 5,891,474), to improve gloss retention of surfactants (U.S. patent No. 5,877,142), as well as for many other purposes.

5. Enzymatic Activity of the Enzymes Produced By the EPS 352 Plasmid

The EPS plasmid contains at least 5 previously unidentified open reading frames encoding 5 previously unidentified enzymes (O, P, N, M, and U, which are provided in SEQ ID NOS: 9, 10, 12, 13, and 14, respectively). Sequence analysis using Blast™ searching indicates that the "M" enzyme (SEQ ID NO: 13) is a glycosyltransferase enzyme. Methods of testing glycosyltransferase activity are

- 26 -

well known in the art and described in: van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741 1996; Kolkman et al., *J. Biol. Chem.* **272**(31):19502-19508; Breton, et al., *Curr. Opin. Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.

Similarly, sequence analysis using BlastTM searching indicates that the "P" enzyme (SEQ ID NO: 10) is a polysaccharide polymerase. Methods of testing polysaccharide polymerase activity are well known in the art and described in: Gonzalez et al., *Proc. Natl. Acad. Sci.* **95**:13477-13482, 1998; Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; and Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993, which are herein incorporated by reference.

Sequence analysis using BlastTM searching indicates that the "N" enzyme (SEQ ID NO: 12) is a galactosyltransferase enzyme. Methods of testing galactosyltransferase activity are well known in the art and described in: van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741, 1996; Kolkman, et al., *J. Biol. Chem.* **272**(31):19502-19508, 1997; Breton et al., *Curr. Opin. Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.

Sequence analysis using BlastTM searching indicates that the "O" enzyme (SEQ ID NO: 9) is a multi-unit transporting or exporter enzyme. Methods of testing activity are well known in the art and described in: Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993; and Smith et al., *Mol. Microbiol.* **4**(11):1863-1869, 1990, which are herein incorporated by reference.

Finally, sequence analysis using BlastTM searching indicates that the "U" enzyme (SEQ ID NO: 15) is a glycosyltransferase/exporter enzyme. Methods of

- 27 -

- testing glycosyltransferase/exporter activity are well known in the art and described in: Stevenson et al., *J. Bacteriol.* **178**(16):4885-4893, 1996; Glucksmann et al., *J. Bacteriol.* **175**(21):7045-7055, 1993; Smith et al., *Mol. Microbiol.* **4**(11):1863-1869, 1990; van Kranenburg et al., *J. Bacteriol.* **181**(1):338-340, 1999; Kranenburg et al., *J. Bacteriol.* **181**(11):6347-6353, 1999; Stingele et al., *J. Bacteriol.* **181**(20):6354-6360, 1999.; Kolkman et al., *J. Bacteriol.* **178**(13):3736-3741, 1996; Kolkman et al., *J. Biol. Chem.* **272**(31):19502-19508, 1997; Breton et al., *Struct. Biol.* **9**:563-571, 1999; and Griffiths et al., *J. Biol. Chem.* **273**(19):11752-11757, 1998, which are herein incorporated by reference.
- 10 Having illustrated and described the principles of the invention in multiple embodiments and examples, it should be apparent to those skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. The invention encompasses all modifications coming within the spirit and scope of the following claims.

CLAIMS

What is claimed is:

5

1. An isolated bacterium having the characteristics of *Lactococcus lactis* subspecies *cremoris* Ropy 352, as deposited with the USDA-ARS-NCAUR-NRRL as deposit accession number NRRL B-30229.

10

2. A purified ropy polysaccharide wherein the polysaccharide has characteristics comprising:

Composition: Glucose: range of 54% to 58%

Galactose: range of 42% to 46%

Charged: Yes

15

Molecular weight: range of 800,000 to 8,000,000

Phosphorous: Present in backbone or sidechain

Structure: endpoints: galactose;
branchpoints: glucose

20

3. A purified ropy polysaccharide, isolated from *Lactococcus lactis* subspecies *cremoris* Ropy 352.

4. The purified polysaccharide of claim 3 wherein the polysaccharide has the characteristics of:

25

Composition: Glucose: range of 54% to 58%

Galactose: range of 42% to 46%

Charged: Yes

Molecular weight: range of 800,000 to 8,000,000

Phosphorous: Present in backbone or sidechain

30

Structure: endpoints: galactose;
branchpoints: glucose

- 29 -

5. A method of thickening a liquid comprising adding to a liquid the purified polysaccharide of claim 2.

6. The method of claim 5 wherein the liquid is a food.

5

7. The method of claim 6 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

10

8. A food product made by the method of claim 6.

9. A method of thickening a liquid comprising adding to a liquid the purified polysaccharide of claim 3.

15

10. The method of claim 9 wherein the liquid is a food.

11. The method of claim 10 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

20

12. A food product made by the method of claim 10.

13. A method of making a food product comprising addition of a culture of Ropy 352 to a food that is devoid of Ropy 352.

25

14. The method of claim 10 wherein the food is selected from the group consisting of milk, a milk-based liquid, a whey-based liquid, a soy-based liquid, and a fruit-juice.

30

15. A food product made by the method of claim 13.

- 30 -

16. An isolated plasmid of approximately 20 kb derived from *Lactococcus lactis* subspecies *cremoris* Ropy 352, wherein the plasmid, when expressed in the transformed lab strain of *Lactococcus* MG1363, expresses a ropy polysaccharide, wherein the polysaccharide has characteristics comprising:

- 5 Composition: Glucose: range of 54% to 58%
 Galactose: range of 42% to 46%
 Charged: Yes
 Molecular weight: range of 800,000 to 8,000,000
 Phosphorous: Present in backbone or sidechain
10 Structure: endpoints: galactose;
 branchpoints: glucose

17. A probe comprising a detectable label attached to a nucleic acid selected from the group consisting of:

- 15 a portion of the plasmid of claim 16, and
 the plasmid of claim 16.

18. A method of detecting a target nucleic acid comprising the steps of:
 contacting the target nucleic acid with the probe of claim 17 under
20 conditions wherein the probe hybridizes with the target nucleic acid, and
 detecting the detectable label.

19. A cell transformed with the plasmid of claim 16.

25 20. The cell of claim 19, wherein the cell is selected from the group
 consisting of: a bacterial cell, a yeast cell, a fungal cell, an animal cell and a plant
 cell.

21. A method of making a food product comprising addition of the cell of
30 claim 16 to a food that is devoid of the plasmid of claim 16.

22. A method for making a pharmaceutical product comprising:

- 31 -

combining an active ingredient and the purified ropy polysaccharide of claim 2.

23. A pharmaceutical product made by the method of claim 22.

5

24. A method of making a beauty care product, comprising adding the purified ropy polysaccharide of claim 2.

25. A beauty care product made by the method of claim 24.

10

26. A method of making a coating agent, comprising adding the purified ropy polysaccharide of claim 2.

27. A coating agent made by the method of claim 26.

15

28. A purified protein, comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOS: 9, 10, 13, 14, and 16;

20 (b) an amino acid sequence that differs from those specified in (a) by one or more conservative amino acid substitutions; and

(c) an amino acid sequence having at least 60% sequence identity to the sequences specified in (a).

25 29. An isolated nucleic acid molecule encoding a protein according to claim 28.

30. An isolated nucleic acid molecule, comprising a nucleic acid sequence selected from the group consisting of:

30 (a) a nucleic acid sequence selected encoding an amino acid sequence selected from the group consisting of: SEQ ID NOS: 9, 10, 13, 14, and 15;

- 32 -

(b) a nucleic acid sequence that shares at least 60% sequence identity with the nucleic acid sequences described in (a);

(b) an nucleic acid sequence that comprises at least 15 consecutive nucleotides of the sequences shown in (b).

5

31. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence according to claim 30.

10 32. A cell transformed with a recombinant nucleic acid molecule according to claim 31.

33. A transgenic bacteria comprising a recombinant nucleic acid according to claim 31.

15 34. A method of producing a protein, comprising:
culturing a cell according to claim 32, wherein the cell expresses at least one protein from the recombinant nucleic acid; and
isolating the protein.

1/15

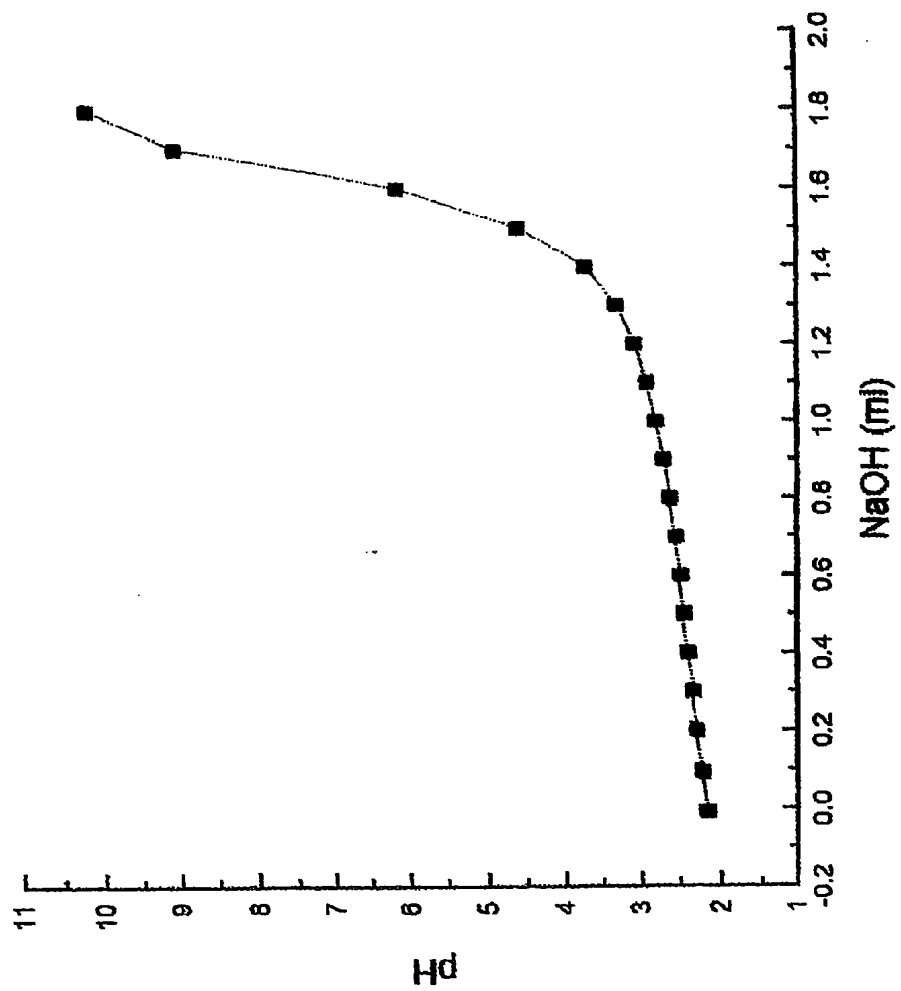


Figure 1

3/15

catcctagcacatactatgtatacaataggcaaaataacttttggttggaataagggttaattactcatacaaatcctttccaccacttagttttgttataaaattcta
 gtaggatcggtgatgatacatatgttatccggttttttatgaaaaccaaactttattccaaaattaatgagttatgttaaggaaaggtgaatcaaaaacaataatttaagat
 V G S C M I H M L S V F M K T K L Y S K L M S M L R K G - I K N N I - D
 ttttaaaaccccaatttttggttaagacaccccaacctgtatgttaatttagatttcgtaaaaattacgctcagaactggcaccagatgccctccctaaactgaagattccttaca
 aaattttggggttaaaaccaaactctgtgggttgacatacatataaatctaaagcatttttaaatcgagctcttgaccgtgtcataggggatttgacttctaagaatgt
 K F W G - N Q F C G L D I H - I - S I F N A S L D R G H R G F D F - E C
 acaattcgtaatgattgctcaatcttaaaatctctgcatttttatagaacactattataaattgaatagttctgtggttttatgacctcaaaattgtccttgaca
 tgttaagcattactaacggggttagaatttttagagagcggtaaaaatatcttctgtgataattattaaacttataagtagcacagacaaaatactggagtttaacaggaactgt
 C - A L L T E L E F - R A - N I L - - L L T Y Q V Q T K I L E F N R N C
 atcttatataaaatatataatcctcatcttatttctacttaggttaattatagtttaataacaagggttatattgttacaaactcttcataataaccatcaaatcattta
 tagaatataattttatataataggagtagaataaagagatgaatccattataatcaattattgttccaatatatacaaatgttgagaagtatatattggtagtttagtaaaat
 - N I I L Y N - E - N K E M N P L I S I I V P I Y N V E K Y I G S L V N
 agagataaactttgttggttcttaaaactcccaataaaataactactgcttagtgactactcttgcaggttttaaaactttctttattaccgtccgtcacttgtt
 tctctattgaaacaaacgaacaagaattttgaggttattttattgtatgacgcatcaactgatgaaagcatgcaaattttgaaagaaataatggcagcagtgaaacaa
 S L L K Q T N K N F E V I F I D D G S T D E S M Q I L K E I M A G S E Q
 cttaaaagcaagttcaacaacgttgttcaattagtcaccaaatagaagtcggtccttatagcccatatgaattacgttgacctcttatatagaaaaaaacctaaagtcta
 gaattttcgttcaagttgtgcaacaagtttaataatcaggggttttatcttcagccaggaatatcggatacttaactaatgcaactggagaatatatcttttttttgattcagat
 E F S F K L L Q V N Q G L S S A R N I G I L N A T G E Y I F F L D S D
 ctactttatcttctgtaaaacacacctctgtttaaactgatcaacgatattttatgtcagttggcctatgtgaatagaaactaatcatcatcgttaactacttaaaccttta
 gatgaaatagaaagcaattttgtgagacaatttttgactagttgtctataaaatcacagtcacccggatacacactatcttggattatagtagcattgatgaatttggaat
 D E I E S N F V E T I L T S C Y K Y S Q P D T L I F D Y S S I D E F G N
 cgaaacctgtcattataaccgtacaccttcataaaatagcaggtttttctaaacacatgttcactcgttttataattgacgtaacagatttctactctatgggttgacgt
 gcttttgacagtaattatggcatggagttattttatctgtcaaaaagatttgtcacagtgagcaaaatatttaactgcatgtctaaaagatgagataccaacaactgca
 A L D S N Y G H G S I Y R Q K D L C T S E Q I L T A L S K D E I P T T A
 accagtaaacattgttttcggagacactaaactttttgtgctaaatgataaaaagacaacaccttttttaaacctctattgttaaaatgocggtttcaaaaaatgaaatca
 tgggtcatttgaacaaaacgctctgtgatttgaaaacacgatttactatttctgttggaaaaaatttggaatacaaatatttacgccggaagtttttttacttagt
 W S F V T K R S V I E K H D L L F S V G K K F E D N N F T P K V F Y F S

Figure 2B

tttttgtaacaacaataaagggtattctaatactatctatatccctttgagagacccagataataactcattagcgggcctttttaagaaaaagcctgctgcggtaaaaacat
 aaaaacattggtgttattccctaagattgtatagatataggaacgcctctgggtctattatgagtaatcgccggaaaaaattcttttcggacgacgcacatttttgtta
 K N I V V I S L R L Y R Y R K R S G S I M S N R P E K F F S D D A I F V
 tgt * atactgaataatctaaaaatactagtcataattttaagcccttaacccctgctcatcaaccatttttatcaatactgttgtaatcgaagaaaaagggtctaagctttt
 aca * tatgacttattagatttttatgacagtataaaaatcgggaattgggagcagtagttggtaaaaatagttatgacaacattagcttcttttccagattcgaaa
 T * Y D L L D F Y D Q Y K I R E L G A V V G K I V M T L A S F P D S K
 ttttaacataattacttaatttaggttagtcttttttcataaaaatttctaataataaagttatctttttctgtatgattgacctattttttacatacatatttttacatacaa
 aaattgtataatgaattaaatccaatcagaaaaaaagtaatttaaaagtattataatttcaatagaaaaaagacatactaaacgggataaaaaatgtatgtaaaaaatgtatgtt
 K L Y N E L N P I R K K V F K D Y I S I E K R H T K R I K M Y V K M Y V
 aaaaagaagaatacaacctatatttgaaatgtctgaccttttccatttgtgaccttcacttatataaaaaattagaataaatac * ISSI insertion
 ttttcttcttatgttgatataaaactttacagactggtaaaaggtaaacactggaactggaagtgaatataaatttttaattcttattatg site
 F S S Y V G Y K L Y R L V K G K H W K - I - F L I L F M

Figure 2C

5/15

B. Alignments
 1) Alignment of EpsM to EpsN

```

EpsM  LSENLSIIIVPVNSEKYLRAAIIHSLLNQTYQNIIEVILINDGSTDGSQELISSFOKKDKR---IKLYNTKNLGVSHARNYGIDRA
EpsN  -MNPLISIIIVPIYNVEKYIGSLVNSLLKQTNKNFEVIFIDDGSTDESMQILKEIMAGSEQEFSFKLLQQVNQGLSSARNIGILNA
      : *****: ** ***: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      : *****: ** ***: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

EpsM  SGSYIMFLDPDDTYDKSYCLEMIGLINKEN-ADVVMNSYYICK--GKNIPNVNNDLLECEGLLSRDKTMRSLSDTGFKGFVWT
EpsN  TGEYIFFLDSDDEIESNFVETILTSCYKYSQPDTLIFDYSSIDFEGNALDSNYGHGSIYRQKDLCTSEQILTALSKDEIPTTAWNS
      : ***: *****: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      : ***: *****: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

EpsM  RIFRKNVINNVKFNESIN-YLEDMLFNISIVHNARIAYTNKRHYFYLOREDSAKKFSKSFKSLNLRGKVDPEFYQSIDSVI
EpsN  FVTKRRSVIEKHDLFLFSVGKKFEDNNFTPKVFYFSKNIVVISLRLYRKRSGSIMSNRPEKFFSDDAIFVTYDLDLDFYDQYKIRE
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

EpsM  FYNLVG-WLITERKSRENSQFIRRNINKNMKSQVKFKTLKMNPIKNLILKLSYAFPLVGSCMIHMLSVFMKTKLYSKLMSMLRKG
EpsN  LGAVVGKIVMTTLASFDPDSEKLYNELNPIRKKVKFDYISIEKRHTKR-IKMY-----VKMYVFSSYVGKLYRLVKGKHKW-
      : ** : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      : ** : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :

```

Figure 3A

Organization of pEPS352

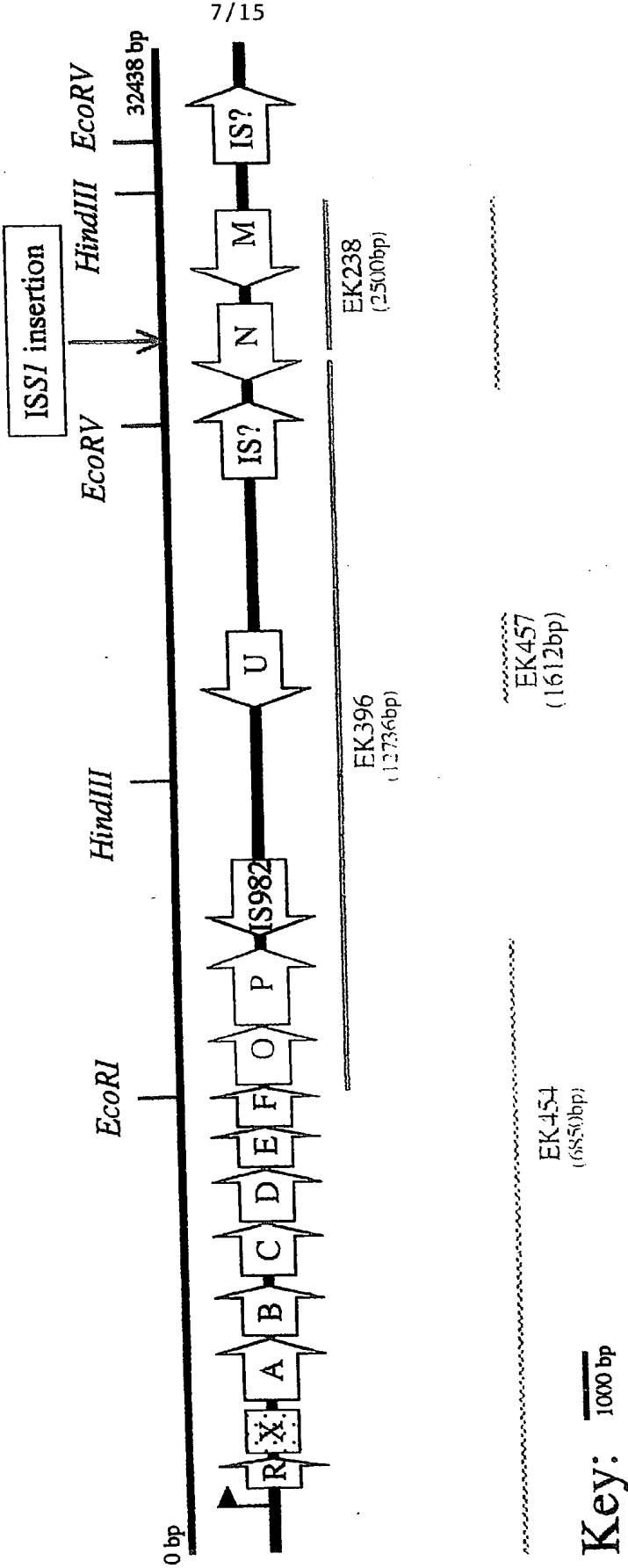


Figure 4

Eps352 Operon sequence EpsR-EpsK (primer EpsOPF-EpsOPR) corrected as of May8, 2000

GTGTAACAAACCCCTACCTTTACTTTGCACATAATAGGTTTTTATTTATATAATATCATTTGATATATATATGAAATTAATAAACAACACCAAAATGGTTTAACTTAAG
CAACTTTTGGGATGGAATGAACGTGATTATCCAAATATAATATAGTAACATAATATTAACCTTTTAACTTTTGTGGTTTACCACAAATGGAATTC
CAAGTTTGTGATTAATTTTTCAGAAAAATTAAGGTTTTTCTACAGAAGTTAAATAAAAAAGSGATTATATTTATGAATAATTTATTTTACCATCGTCTA
GTTCAAAACATAAAATTAAGAGTCTTTTATATCCAAAAAGAAATGCTTCAATATATTTTTTCCCTAATATAATAATACCTATTATAAAATACTATTATAAAATAATGGTAGCAGAT
M N N L F Y H R L
AAGGAACCTAGTGAATCAAGTGGTAAATCTGCAAATCAAAATGAAGGGAATGGGTACCCATAGAAATCTCTTGAATAATATTAAGTGGGAGGAGAAC
TTCCCTTGATCAACTTAGTTCCACATTTAGACGTTAGTTTATCTTCCCTTAACCCAATGGGATCTTTAAGAACTTATTAATATTAACAACCCCTCCCTCTTG
K E L V E S S G K S A N Q I E R E L G Y P R N S L N Y K L G G E
CCTCTGGGACAAAGATTAATAGGACTATCAGAGTATTTTAATGTCTCTCCAAATATCTGATGGGTATAATTTGATGAGCCTAATGACAGTCTGCAATTA
GGAGACCCCTGTTCTTAATTAATCTCTGATAGTCTCAATAAATTACACAGAGGTTTTATAGACTACCCATATTAACCTACTCCGGATTACTGTCAAGACGTTAAIT
P S G T R L I G L S E Y F N V S P K Y L M G I I D E P N D S S A I N
TCCTTTTAAACCTAACTCAAGAAGAGAAAAAGAAATGTTTATAATTTGTCAAAAATGGCTTTTTTAGAATATCAAAATAGAGTTATAACAATAATAA
AGAAAAATTTTGAGATTGAGTTCTCTCTCTTTTCTTTTACAAATATTAACAGTTTTTACCGAAAAAATCTTATAGTTTATCTCAATATGTTATTATT
L F K T L T Q E E K E M F I I C Q K W L F L E Y Q I E L
ATTAGGGAGTTTTTTCGGTAGTGAAAAATAAGTTTTGGAACATCAAAAAATATCACTACAAATGGCGAAACAAGTGAACAATATTATGGCTGAAAAAGTTC
TAATACCTCAAAAAAGCCATCACATTTATTCAAAACCTTGATGTTTTTATAGTGGATGTTACCGTTTTGTTCACTCTGTTAATAAACCGACTTTTTCAG
N K F W N I K N I T Y N G E T S E Q L L A E K V
AAAAATCAAGTATTGGCGACTAACCCCTGATGTTGTTTTTATATGAAGCTCCACTTTTTTAATGATAACCAAAACATTGAAGCAACAGCCTCATGGACTAGTAA
TTTTTAGTTCATAACCGCTGATTGGGACTACAACAAATATATCTTCGAGGTGAAAAATTAATCTATTGTTGTTGTAACCTCTGTCGGAGTACTGATCATTT
Q N Q V L A T N P D V V L Y E A P L F N D N Q N I E A T A S W T S N
TGAGCAACTTATAACAAATTTGGCTAGTACAGGACGAGGTGATAGTCTCAACCCCTCTCCACCGATTATATGTTGTTGTACCCCGTACAAGAAGAA
ACTCGTGAATATTGTTTAAACCGATCATGTCTCTCGTCTCCACTATCAAGTTGGAGAGGTGGCTAAATACCCACACACATATGGGCGATGTTCTTCTT
E Q L I T N L A S T G A E V I V Q P S P P I Y G G V Y P V Q E E
CAGTTTAAACAATCTTTATCTACAAGTATCCCTATATAGACTACTGGCTAGTTACCCAGACAAAAATTTCTGATGAATGAAGGGGCTGGTTCTGTGATG
GTCAAAATTTGTTAGAATAAGATGTTTCATAGGATATATCTGATGACCCGATCAATGGTCTGTTTTTAAAGACTACTTTTACTTCCCGACCAAGACTAC
Q F K Q S L S T K Y P Y I D Y W A S Y P D K N S D E M K G L V S D

Figure 5A

9/15

ATGGAGTATATAGAACATTAAATGCTTCGGGAAATAAGGTTTGGCTAGATTATATACCTAAATATTTTACAGCAAACTAAATTAAGTTTATAAATAACAATTT
TACCTCATATATCTTGTAATTTACGAAGCCCTTATTCAAAACCGATCTAAATAATGATTTATAAAATGTGCTTGATTAATTCAAATATTTATTTGTTTAA
D G V Y R T L N A S G N K V W L D Y I T K Y F T A N
ATTAAATATTGGAGAAGAAATCCAGGAAACACAGGAAACAGACGATTGATTTTAAGAGGGATTTTAAATATTTTCGCCAAAAGGTTAGGTTTAATATATTATT
TAATTTATAACCTCTCTTTTAGCTCTTTGTGTCCTTTGTCTGTAACATAATTCCTCCCTAAAATTTAAATTAAGCGTTTCCAAATCCAAATTAATAATAAA
M Q E T Q E Q T I D L R G I F K I I R K R L G L I L F
AGTGCTTTAAATAGTCACAAATATAGGAGCATCTACACATTTTATAGCCTCCCGAGTTTACAGAGCCTCAACTCAACTTGTCTGTTAAACTACCAAAT
TCACGAAATATCATAGTGTATAATCCCTCGTAGATGTGTAATAATATCGAGGGGTCAATGTGTCGGAGTTGAGTTGAACAGCAATTTGATGGTTTAA
S A L I V T I L G S I Y T F F I A S P V Y T A S T Q L V V K L P N N
CGGAGCATTCAGCAGCCTACGCTGGAGAAGTACCGGGAAATATCAAAATGGCGAACACAAATTAACCAAGTTATTGTTAGTCCAGTCATTTTAGATAAAAGT
GCCTCGTAAGTCGTCGGATGCGACCTCTTCACCTGGCCCTTATAAGATTACCGCTGTGTTAATTTGTTCAATAACAAATCAGGTGAGTAAATCTATTTC
S E H S A A Y A G E V T G N I Q M A N T I N Q V I V S P V I L D K V
TCAAAAGTAATTTAAATCTATCTGATGGCTCTTTCCAAAACAAAGTTACAGTAGCRAATCAACAGATTCACAAAGTTATTACGCTTACTGTTAAATATCTT
AGTTTCATTAATTTAGATAGACTACCGAGAAAGGTTTTTGTTCATGTCTATCGTTAGTTTGTCTAAGTGTTCAAATATCGGAATGACAAATTTATAAGA
Q S N L N L S D G S F Q K Q V T V A N Q T D S Q V I T L T V K Y S
AATCCTTACATTCACAAAAGATTGCAGACGAGACTGCTAAAATTTTGTAGTTTCAGATGCAGCAAACTATTGAATGTTACTAACTGTTAAATTTCTATCTCA
TTAGGAATGTBACGTGTTTTCTAACGTCCTGCTCPCAGCATTTTAAAAATCAAGTCTACGTCGTTTTCGATAAATTCACAAATGCAATTTAATAAGATAGGT
N P Y I A Q K I A D E T A K I F S S D A A K L L N V T N V N I L S
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TTGCTTTTCGAGTTGTTGTGTTAATCAGGATTTGGATTAAACATAAATCGCTATAGACAAATATCGGCTGTATCAAAATCCAAATCGGTAAACGAAATAA
K A K A Q T T P I S P K P K L Y L A I S V I A G L V L G L A I A L L
GAAGGAATATTGATAACAAAATTAATAAGAGAAGATATTGAAGCTCTGGGCTCACGGTTCTTGGTGTAACAAGCTATGCTCAAAATGAGTGAATTT
CTTCCCTTAATAAACTATTGTTTAAATATTCTTCTCTATAACTTCGAGACCCGAGTGCCAAAGAACACATTTGTCGATACGAGTTTATCTCACTAAAA
K E L F D N K I N K E E D I E A L G L T V L G V T S Y A Q M S D F
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N K N T N K N G T Q S G T K S S P P S D H E V N R S S K R N K R
AGTTCAGGATGGCTAAAAATAAAGAAGCATAGACAACAATCGTTATATTACCAGTGTCAATCCCAATCACCTATTTCCGCAACAATATCGTTCGAT
TCAAGTCTACCGATTTTATTTTCTTCGTATCTGTTGTAGCAATATAAATAGTGCACAGTTAGGAGTAGTGGATAAAGCTTGTATTATAGCAAGCTA
M A K N K R S I D N N R Y I I T S V N P Q S P I S E Q Y R S I
TCAAGTCTACCGATTTTATTTTCTTCGTATCTGTTGTAGCAATATAAATAGTGCACAGTTAGGAGTAGTGGATAAAGCTTGTATTATAGCAAGCTA

Figure 5B

Figure 5C

AAGAACTTTTATAAATATTCAATTGGAGGACTCAACCTATTTTGGTCCACCTGAGCCGTAATAGCGGAATCATTTGAAACCCCTGATATATTATTGA
TTCTTGCAAAAAAATATTATAAGTTAACTCCCTGAGTTGGATAAAACAGGTGGGACTCGCATATTCGCCCTTAGTAACCTCTGGGACTATATAATAAACT
K E L F Y N I Q L E G L Q P I L V H P E R N S G I I E N P D I L F D
TTTTATTGAACAAGGAGTACTAAGTCAGATAACAGCTTCAAGTGTCACTGTGCATTTTGGTAAAAAAAATACAAAAGCTGTCTATTTAAATGATAGAAAAAC
AAAAATACTTGTTCCTCATGATTCAGTCTATTGTGCAAGTTTCACAGTGACCACTAAAACCACTTTTATTATGTTTCGACAGAPAAATTTTACTATCTTTTG
F I E Q G V L S Q I T A S S V T G H F G K K I Q K L S F K M I E N
CATCTTACGCAATTTTGTTCATCAGATCGCATATGTGACGTCACTGTCATTTAAGATGAAGGAAGCGTTTGAAATATTATTGAAGATAGTTATGTTCTG
GTAGAATCGGTAAAAACAACAGTAGTCTACGCGGTATTACACCTGCAGTGCACGTAAATCTACTTCTCGCAAACTTTAACTCTCTATCAATACCAAGAC
H L T H F V A S D A H N V T S R A F K M K E A F E I I E D S Y G S
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TACATAGTGCTTACAAAGTTTATTAGCTCTCAGTCACTAAAATTTGCTTTCAAAAATAGTTCTTTTGGTTGTTCTAGTTTGTCTTTTAAAAAATCC
D V S R M F Q N N A E S V I L N E S F Y Q E K P T K I K T K K F L G G
ATTATTTTAAAAAGGATTAAGAAGGATTAATAATGGAATTTTGTGAGGATCGCTCATCACCTGAATCGGAGAGCGCTAAGTTAGTAGAAATTAATAAAATTTT
TAATAAAATTTTCCCTCAATTTTCCCTCAATTTTACCCTAAAAACCTCTACGGAGTAGTGAGCTAGCCCTCTCGGATTCATCATCTTAATTTTAAAA
L F M E F F E D A S S P E S G E P K L V E L K N F
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S Y R E L I I K R A I D I L G G L A G S V L F L I A A A L L Y I P
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TGTTTTCACGAGTTTCTAGTTCGCGTTACAGATATTTGTTTGGGATACCATTTTACCATTTTAAAAAATATAAACTTTTAAATCTTAAATCTTGTGTA
Y K M S S K K D Q G P M F Y K Q K R Y G K N G K I F Y I L K F R T M
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CTAAGAAATTACGGCTCGTCAATAGATCTTGAATTAGGTCATACAAATTCGACGAATGGTACGGTTGCGGTATTTCGATCTTTTGCAGGTGCCCATCTGCTC
I L N A E Q Y L E L N P D V K A A Y H A N G N K L E N D P R V T K
ATTGGCTAATTATAAGACGACACTCAATTGATGAACTGCCACAATTTATCAATGTCTTAAAGGGGATATGCTAATGTTGGTCCAAGACCAATTTCTG
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I G S F I R H S I D E L P Q F I N V L K G D M S L V G P R P I L L
TTTTTGAAGCGAAAAGAATATGGGAACGCTCGCTTACTACTCATGTGCAACACGGAATCAGCTGGTTATTGGACGACACATGTCGGAAGTAAAGTTCT
AAAAACTTCGCTTCTTATACCTTTGCGAGCGGAATGAATGAGTACACGTTTGGTCCCTTAGTGACCAATAACCTGCTGTGTACCAGCTTCATTTCAAGA
F E A K E Y G K R L A Y L L M C K P G I T G Y W T T H G R S K V L

11/15

Figure 5D

12/15

TTTTCCCTCAACGAGCAGATTAGAACTCTATTATCTCCAGTACCATAGCACCAAAATGATATCAAGCTTCTACTACTCACAATTTGTACAAAGTATTAAC
 AAAAGGAGTGTCTCGTCTAAATCTTGAGATAATAGAGGTCTATCGTGGTTTTACTATAGTTCGAAGATCATGAGTGTAAACATGTTTTCATATAATG
 F P Q R A D L E L Y Y L Q Y H S T K N D I K L L V L T I V Q S I N
 GGATCGGCGCATATTAAAAATGAAAATAGCATTTAGTGGTCCAGCGGTGGCCATTGACACACCTGTATTGTTAAAAAGTTTGGGAAAACGAAG
 CCTAGCCTCGGTATAATTTTACTTTTATCGTAATCATCCAAAGTCGCCACCGGTAAACTGTGGACATAAACAAATTTTTCAAAACCCCTTTTGCTTC
 G S D A Y M K I A L V G S S G G H L T H L Y L L K K F W E N E
 ATAGATTTTGGGTCACATTGATAAAACAGATGCAAAATCTATATTGAAAGAGAAAGATTTTATCCTTGTATTATCCACAAAATAGAAAATGTAATAAA
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 D R F W V T F D K T D A K S I L K E E R F Y P C Y Y P T N R N V K N
 CACGATAAAAAATACCATTTCTGCAATTTAAAAATACCTAGAAAAGAAAACCAAGATTGATTATTTTCGAGTGGTGTGCGGTAGCCGTTCCTTTTTTGG
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 T I K N T I L A F K I L R K E K P D L I I S S G A A V A V P F F W
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 L G K L F G A K T V Y I E I F D R I D K P T L T G K L V Y P V T D
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 TCAAAATATCAAGTTACCTTCTCAATTTTTCAAATGGGATTTTCGTTAATTAATCCTCCTTAAAGATTACTAAAACATTGCCAACCTTGAGTGT
 K F I V Q W E E L K K V Y P K A I N L G G I F M I F V T V G T H E
 CAACCATTTAATCGACTCATCAAAAAATGTGAACTGTACCGGTGGTGAATCGAAGACGATGATTTCATGCAAAATGGGTACTCAACTTATGAAC
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 Q P F N R L I Q K I D E L V R D G E I E D D V F M Q I G Y S T Y E
 CTAAATATACFAAAATGGGAAAAGTTTATGGATATGAGACTATGGAAGATGTATGAATGAAGCGAGTACGATTATTACTCATGGCGACCATCTACCTA
 GATTATATGATTTACCTTTTCAAAATACCTATACCTGTATACCTTTCTACATACCTTCTGCTCATGCTAATAATGAGTACCGCTGGTAGATGGAT
 P K Y T K W E K F I G Y E T M E R C M N E A S T I I T H G G P S T Y
 TATGCAAGTATTACAACCTAGGTAAAAATTCOGATAGTTGTTCACGGGAAATGAAATTTGATGAGCATATAAATGATCATCAACTTTGGGTAAAGTAAACAG
 ATACGTTCAATATGTTGATCCATTTTAAAGGTATCAACAGGTGCCGTTACTTTTAACTACTCGTATATTACTAGTAGTTGAACCCATTCATTCTGTC
 M Q V L Q L G K I P I V V P R Q M K F D E H I N D H Q L W V S K Q
 GTGTGAAAAAGGGATACCTATTGATTTTGTGCGAAGATGTGAAGACATCTCGAAAATATTATTAGTTCAAAATTCAGATACCTTACAAAATAATG
 CAACACTTTTCCCTATGAGTAACATAAACACGCTTCTACAACCTTCTGTAAGAGCTTTTATAATAATCAAGGTTTTTAAAGTCTATGGAATGTTTTTAC
 V V K K G Y S L I L C E D V E D I L E N I I S S K I S D T L Q K N

Figure 5E

13/15

TAAATCACAACACTGAATTCATAAAATATTTCAGTGTGCTGAAATTTACCAGCTATTATATAAAAGTGAGAAGATATGATACCAAAGTAATACACTATTGC
 ATTTAGTGTGTGACTTAAAGTATTTTAAATAAGTCACGACTTTAAATGGTCGATAAATATTTTTCACCTCTCTATACCTATATGTTTTCATTTATGTAFAACG
 V N H N T E F I K L F S A E I Y Q L F I K S E K I M I P K V I H Y C
 TGGTTCGGAGGCAACCTTTACCAGAACTCGCTAAATGTATTGAAAGTTGGAGAAGGTTTGTCCAGATTATGAAATAAAACAAATGGTCTGAGAAAA
 ACCAAGCCCTCCGTTGGAATGGTCTTAGACGCGATTATACATAACTTTCAACCTTCCAAAACAGGCTCAATACCTTTATTTTGTACCAGACTCTTTT
 W F G G Q P L P E S A L K C I E S W R F E C P D Y E I K Q W S E K N
 ACTATGATGTAATAATAAATTCATAATTAAGGAAGCATATCAAGAAAAAATTTTGTCTTGTACGGATGTTGCAAGGCTCGATATAATTTGGAATGA
 TGATACTACATTTATTTAAGTTATATAATTCCTTCGTATAGTTCTTTTAAACGAAAAACAGTGCTACAACTGTCGAGCTATATATAACCTTACT
 Y D V N K I Q Y I K E A Y Q E K F A F V T D V A R L D I I W N E
 AGCGGGTATATATCTTGACACGGATGTAGCTTATAAATCTCTTGATGAATTTGCTGTATAATAGTTTATATTTAGGAATGGAAGAGCTGGTAGAGTA
 TCCGCCATATATAGAACTGTGCTACATCTCGAATATTTTAGAGAACTACTTAACGACATATATCAAAATATAATCCCTTACCTTTCTCGACCATCTCAT
 G G I Y L D T D V E L I K S L D E L L Y N S L Y L G M E R A G R V
 AATACGGGTTTAGGGTTTGGAGCTGAAGTAAATCATCCAAATGTGAGAGCTAATTTAGATTGTATACHTATATTCCTTTTCAGGCAATGATAATATAA
 TTATSCCAAATCCCAACCTCGACTTCATTAGTAGGTTAACTCTGATTAATCTTAACATATGATATAAGGAAAAAGTCCGTTACTATTATATT
 N T G L G F G A E V N H P I V R A N L E L Y T N I P F S G N D N I T
 CTGTGTGACCTATACGACGAATCTTTTGAAAAAATATGCTCAAAAAACAACAAATGAATCAACATATAGATAACGCAATAATTTTACCTACTGAATA
 GAACACACTGGATGCTGCTTAGAAAACTTTTATACCAAGATTTTGTGTACTTTTAAGTTGTATCTATTTGCGTTATTAAATGGAATGACTTAT
 C V T Y T T N L L K K Y G L K N N N E I Q H I D N A I I L P T E Y
 TTTATGTCCTCTAAGTTTGAACAAATCGATTAAAAATAACGAAAAATACCTACTCCATCCATCATGATATGAGTTGGAAAAAGATAAGAGAGATAAA
 AATACAGGAGATTCAAAACCTTTGTGTAGCTAATTTTATTTGCTTTATGATGAGGTAGGTAGTACTATACCTCAACCTTTCTATTCTCTCTATT
 L C P L S F E T N R L K I T E N T Y S I H Y D M S W K D K R D K
 TTTTAAAGACTTAAATACAACTTAGAAAAATGGGTAGGTGATGATTTTATGAAAAAGTTTATPAAAAAGAAATGGAATAATATATCATGAATPAAAAAATAC
 AAAAAATCTGAAATTTTATGTTGAATCTTTTACCCATCCACTACTAAAAATACCTTTTCAATAATTTTCTTAACCTTTTATTAATAGTACTTATTATTATG
 F L R L K I Q L R K W V G D D F Y E K V I K R I G K M N K I T
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 GTACTGTTCTCTACTCTCAATAACGGAATACACGCAATTAATCTTATAAATTTATATGCTTAATTAACGCGAGAAAGTCGTATGAGAAAAATCGTAC
 M T R E M R V I A L C V V I L E Y L N N T G L I A S S A Y S F S M
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 A S T I L L S Y I L F C K K R K G F S L K E I I V L L I P F I F V

Figure 5F

14/15

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 AAAATTTAGCACTAGGATCATTTAAAGTCAAAATCCCAATACACCTATCAGATAAAATACAAATTCATTTCAGCCTTTATCTAAATTTTTTTCACCTACTTTTG
 V L N R D P S N F S L G L M W I L Y F M L S K S E I D L K K V M K T
 ATTTTGTGTTACCTCTAGTGTGTTGTTTATTTTGACAATAGTACTTTTATTAATAATGTCCTTAATAAAAGCTCGATATGATATGATGCGGTGGAGAT
 TAAAAACAATGGAGATCACAAACAAAAATAAACTGTTATCATGAATAAATATTATACAGAGAATATTATTCGAGACTATACTATTACACCGCACCTCTA
 F F V T S S V C F I L T I V L Y L I M S L N K S S D M I M W R G D
 GCTTTTATAAATCGTATGAGTTTAGGATTTATCCAAACCGAATTTTGCAATGATGAGCTTTTATAGGTATAGCGATAGCCTTATTATATTGAGTACTGAAA
 CGAAAAATATTAGCATACTCAAAATCCATAAATAGGTGGAATAAAACGTTACTACTCGAAAAATCCATATCGCTATCGGAATAATATAAACTCATGACTTT
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 R Q R I T I I F I A I V T F I I F Y F T Q S R T S G Y I L F I L S
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 ATAAAAATAACAATCATCATCTTTTGTGATTTTTCGTTCAAGTTTAAACITTTTCTCGTAATGTCAAAAATGGTGATGAAGAAAAATAGTAGAGAATA
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 AGCAACAATTTCAATGGATAATTAGTTATGTAGTTATCGAACCAAGACCGAGACCGCGAAATAGTCTCTAAATAAGATGTAAACCATATGTAAGTAACT
 S L L K L P I N Q Y I N S L L S G R L A L Y Q E I Y S T F G I H L
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 ATCCCTTATTACTACAAATTTTATGTTTACATCTATGTCGATAGAAAGTTTCAACGATCGTTTTTCCITAAAAACAATGTAAACAAAAATAAACATTGAAA
 I G N N D V K N T M L D T A Y L Q S L L A K G I L F T L F L F V T F
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 GAAAAAGTATAAAAAAGATTCTCTTTTTTGTGTTGATCCAACTTTCAAAATCAATTAATACTACATAAAAAATTAACGTAATGCTTTGTAGTAAAAAA
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 TCCAAACATTAATAAAGGTCAATAACTACCATATTACCTAGCTTTCTCCGATTATTTCATTATCTTTTCCACCGTATCCTCATATAATTATTGTCI
 R F V I L F P V L M V I M D Q K E A N K V I E K V A
 GATTGAGGAATACAAAGTATCCGTTATAGTTCCCTGTTTACAATGTAGAGG
 CTAACCTCTCTGTTTCATAGGCAATATCAAGGACAAATGTTTACATCTCC

Figure 5G

15/15

Sequence of EpsU (start and stop codons are underlined) 1612bp total
here but 1412 from start codon to stop codon

GGTGGACAGGAGGACACAATTTTAAATCCTTCCTGTTATATAGTTTTTGTTTAATATTTTTCGGGAGGGTT
ATTAATGCAAATCGCAAAAAATTATCTTTATAATGCAATATATCAGGTCTTTATAATAATTGTGCCATTAC
TTACCATTCCTTATTTGTCAAGAATTTTGGGCCCTTCAGGTATTGGAATTAACATACCAATTCTATT
GTCAGTATTTTGTCTTATTTGGTAGTATAGGAGTCGGTTTGTATGGGAATCGTCAGATTGCCTTTGTAG
GGATAATCAGGTCAAATGCTCTAAAGTCTTTTATGAAATATTTATTTAAGACTATTTACAATATGTTTAG
CATATTTTTTGTTCGTTGCTTTTTTAATCATTAATGGTCAGTATCATGCATACTATTTGTCTCAATCCATT
GCTATAGTTGCAGCTGCATTTGATATCTCTTGGTTTTTATGGGAATTGAAAATTTTAAAGTAACTGTATT
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GCTCCGGCTTTTTTGATCAGTCTGATAAAATAGTTAACTGGTTTTGGCTATTGCTACTGCAACAGGTACT
GTCATGTTGCCACGTGTTGCAAATGCCTTTGCACATAGAGAGTATAGTAAATTAAGGAATACATGTACGC
AGGTTTTTCTTTTGTGTCGGCAATTCGATTCCCTATGATGTTTGGTCTGATAGCTATTACTCCTAAATTCG
TGCCACTTTTTTTTACATCTCAATTTAGTGATGTTATTCCTGTGTTAATGATCGAGTCAATCGCAATTATT
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CTTTAAATTAGTTTGTGTTAACACCGACATCTTGATATTCAATCTGTTGGAAATTACTGTGGGCATAATTA
TTTATGTTGTTTTATTAATATTTTTTAAAGGCAGAAATAATTAATAAGCTAAAGTTTATTATGCATAAAATAG
AGGTATGGATTTAGGTACCTGCCTTATTGAAAATAACGGTGAGTCAATGGTATTGGGCATATTTGACGCTC
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Figure 6

SEQUENCE LISTING

<110> Trempy, Janine, et al.

<120> BIOPOLYMER THICKNER

<130> 58153

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<150> 60/241,098

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cagaaaaatt aaggtttttc ttacagaagt taataaaaaa agggattata ttt atg      176
                                     Met
                                     1

aat aat tta ttt tac cat cgt cta aag gaa cta gtt gaa tca agt ggt      224
Asn Asn Leu Phe Tyr His Arg Leu Lys Glu Leu Val Glu Ser Ser Gly
      5                                10                                15

aaa tct gca aat caa ata gaa agg gaa ttg ggt tac cct aga aat tct      272
Lys Ser Ala Asn Gln Ile Glu Arg Glu Leu Gly Tyr Pro Arg Asn Ser
      20                                25                                30

ttg aat aat tat aag ttg gga gga gaa ccc tct ggg aca aga tta ata      320
Leu Asn Asn Tyr Lys Leu Gly Gly Glu Pro Ser Gly Thr Arg Leu Ile
      35                                40                                45

gga cta tca gag tat ttt aat gtg tct cca aaa tat ctg atg ggt ata      368
Gly Leu Ser Glu Tyr Phe Asn Val Ser Pro Lys Tyr Leu Met Gly Ile
      50                                55                                60                                65

att gat gag cct aat gac agt tct gca att aat ctt ttt aaa act cta      416
Ile Asp Glu Pro Asn Asp Ser Ser Ala Ile Asn Leu Phe Lys Thr Leu
      70                                75                                80

act caa gaa gag aaa aaa gaa atg ttt ata att tgt caa aaa tgg ctt      464
Thr Gln Glu Glu Lys Lys Glu Met Phe Ile Ile Cys Gln Lys Trp Leu
      85                                90                                95

ttt tta gaa tat caa ata gag tta taa caataataaa tttagggagt      511
Phe Leu Glu Tyr Gln Ile Glu Leu
      100                                105

tttttcggta gtgtaa aat aag ttt tgg aac atc aaa aat atc acc tac aat 563
                        Asn Lys Phe Trp Asn Ile Lys Asn Ile Thr Tyr Asn
                        110                                115

ggc gaa aca agt gaa caa tta ttg gct gaa aaa gtt caa aat caa gta      611
Gly Glu Thr Ser Glu Gln Leu Leu Ala Glu Lys Val Gln Asn Gln Val
      120                                125                                130

ttg gcg act aac cct gat gtt gtt tta tat gaa gct cca ctt ttt aat      659
Leu Ala Thr Asn Pro Asp Val Val Leu Tyr Glu Ala Pro Leu Phe Asn
      135                                140                                145                                150

gat aac caa aac att gaa gca aca gcc tca tgg act agt aat gag caa      707
Asp Asn Gln Asn Ile Glu Ala Thr Ala Ser Trp Thr Ser Asn Glu Gln
      155                                160                                165

ctt ata aca aat ttg gct agt aca gga gca gag gtg ata gtt caa ccc      755
Leu Ile Thr Asn Leu Ala Ser Thr Gly Ala Glu Val Ile Val Gln Pro
      170                                175                                180

tct cca ccg att tat ggt ggt gtt gtg tac ccc gta caa gaa gaa cag      803
Ser Pro Pro Ile Tyr Gly Gly Val Val Tyr Pro Val Gln Glu Glu Gln
      185                                190                                195

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agt tac cca gac aaa aat tct gat gaa atg aag ggg ctg gtt tct gat	899
Ser Tyr Pro Asp Lys Asn Ser Asp Glu Met Lys Gly Leu Val Ser Asp	
215 220 225 230	
gat gga gta tat aga aca tta aat gct tcg ggg aat aag gtt tgg cta	947
Asp Gly Val Tyr Arg Thr Leu Asn Ala Ser Gly Asn Lys Val Trp Leu	
235 240 245	
gat tat att act aaa tat ttt aca gca aac taattaagtt ataaataaca	997
Asp Tyr Ile Thr Lys Tyr Phe Thr Ala Asn	
250 255	
attattaaat attggagaag aa atg cag gaa aca cag gaa cag acg att gat	1049
Met Gln Glu Thr Gln Glu Gln Thr Ile Asp	
260 265	
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Leu Arg Gly Ile Phe Lys Ile Ile Arg Lys Arg Leu Gly Leu Ile Leu	
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Phe Ser Ala Leu Ile Val Thr Ile Leu Gly Ser Ile Tyr Thr Phe Phe	
285 290 295	
ata gcc tcc cca gtt tac aca gcc tca act caa ctt gtc gtt aaa cta	1193
Ile Ala Ser Pro Val Tyr Thr Ala Ser Thr Gln Leu Val Val Lys Leu	
300 305 310	
cca aat tcg gag cat tca gca gcc tac gct gga gaa gtg acc ggg aat	1241
Pro Asn Ser Glu His Ser Ala Ala Tyr Ala Gly Glu Val Thr Gly Asn	
315 320 325 330	
att caa atg gcg aac aca att aac caa gtt att gtt agt cca gtc att	1289
Ile Gln Met Ala Asn Thr Ile Asn Gln Val Ile Val Ser Pro Val Ile	
335 340 345	
tta gat aaa gtt caa agt aat tta aat cta tct gat ggc tct ttc caa	1337
Leu Asp Lys Val Gln Ser Asn Leu Asn Leu Ser Asp Gly Ser Phe Gln	
350 355 360	
aaa caa gtt aca gta gca aat caa aca gat tca caa gtt att acg ctt	1385
Lys Gln Val Thr Val Ala Asn Gln Thr Asp Ser Gln Val Ile Thr Leu	
365 370 375	
act gtt aaa tat tct aat cct tac att gca caa aag att gca gac gag	1433
Thr Val Lys Tyr Ser Asn Pro Tyr Ile Ala Gln Lys Ile Ala Asp Glu	
380 385 390	
act gct aaa att ttt agt tca gat gca gca aaa cta ttg aat gtt act	1481
Thr Ala Lys Ile Phe Ser Ser Asp Ala Ala Lys Leu Leu Asn Val Thr	
395 400 405 410	
aac gtt aat att cta tcc aaa gca aaa gct caa aca aca cca att agt	1529
Asn Val Asn Ile Leu Ser Lys Ala Lys Ala Gln Thr Thr Pro Ile Ser	
415 420 425	

cct aaa cct aaa ttg tat tta gcg ata tct gtt ata gcc gga cta gtt	1577
Pro Lys Pro Lys Leu Tyr Leu Ala Ile Ser Val Ile Ala Gly Leu Val	
430 435 440	
tta ggt tta gcc att gct tta ttg aag gaa tta ttt gat aac aaa att	1625
Leu Gly Leu Ala Ile Ala Leu Leu Lys Glu Leu Phe Asp Asn Lys Ile	
445 450 455	
aat aaa gaa gaa gat att gaa gct ctg ggg ctc acg gtt ctt ggt gta	1673
Asn Lys Glu Glu Asp Ile Glu Ala Leu Gly Leu Thr Val Leu Gly Val	
460 465 470	
aca agc tat gct caa atg agt gat ttt aat aag aat aca aat aaa aat	1721
Thr Ser Tyr Ala Gln Met Ser Asp Phe Asn Lys Asn Thr Asn Lys Asn	
475 480 485 490	
ggc acg caa tcg gga act aag tca agt ccg cct agc gac cat gaa gta	1769
Gly Thr Gln Ser Gly Thr Lys Ser Ser Pro Pro Ser Asp His Glu Val	
495 500 505	
aat aga tca tca aaa agg aat aaa aga tag gagttcagg atg gct aaa aat	1820
Asn Arg Ser Ser Lys Arg Asn Lys Arg Met Ala Lys Asn	
510 515 520	
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Lys Arg Ser Ile Asp Asn Asn Arg Tyr Ile Ile Thr Ser Val Asn Pro	
525 530 535	
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555 560 565	
gaa gta gct gta ggt aaa tca acc gta tgt gct aat ata gct gtt gct	2012
Glu Val Ala Val Gly Lys Ser Thr Val Cys Ala Asn Ile Ala Val Ala	
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Lys Pro Thr Val Asn Ile Thr Phe Lys Val Gln Asn Arg Val Gly Leu	
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Thr Asn Ile Leu Met His Gln Ser Ser Ile Glu Asp Ala Ile Gln Gly	
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gca gtt att gtt gta cgt gcc tat gaa aca aaa aaa gag agt tta gca	2396
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Ile Thr Ala Thr Pro His His Asn Pro Gln Phe Asn Asn Glu Ser Pro	
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780 785 790	
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Gln Leu Pro Ile Glu Val Leu Pro Gly Gln Glu Val Arg Ile Tyr Gly	
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Ser Ser Val Thr Gly His Phe Gly Lys Lys Ile Gln Lys Leu Ser Phe	
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Lys Met Ile Glu Asn His Leu Thr His Phe Val Ala Ser Asp Ala His	
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Asn Val Thr Ser Arg Ala Phe Lys Met Lys Glu Ala Phe Glu Ile Ile	
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gaa gat agt tat ggt tct gat gta tca cga atg ttt caa aat aat gca	3229
Glu Asp Ser Tyr Gly Ser Asp Val Ser Arg Met Phe Gln Asn Asn Ala	
940 945 950	
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Glu Ser Val Ile Leu Asn Glu Ser Phe Tyr Gln Glu Lys Pro Thr Lys	
955 960 965	
atc aaa aca aag aaa ttt tta gga tta ttt taa aaggattaaa aggagtaa	3330
Ile Lys Thr Lys Lys Phe Leu Gly Leu Phe	
970 975	
a atg gaa ttt ttt gag gat gcc tca tca cct gaa tcg gga gag cct aag	3379
Met Glu Phe Phe Glu Asp Ala Ser Ser Pro Glu Ser Gly Glu Pro Lys	
980 985 990 995	
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Leu Val Glu Leu Lys Asn Phe Ser Tyr Arg Glu Leu Ile Ile Lys Arg	
1000 1005 1010	
gca att gat atc cta gga gga tta gca ggt tca gtt tta ttt ctt att	3475
Ala Ile Asp Ile Leu Gly Gly Leu Ala Gly Ser Val Leu Phe Leu Ile	
1015 1020 1025	
gcg gct gca ttg ctt tat atc cct tac aaa atg agc tca aaa aaa gat	3523
Ala Ala Ala Leu Leu Tyr Ile Pro Tyr Lys Met Ser Ser Lys Lys Asp	
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caa ggg cca atg ttc tat aaa caa aaa cgc tat ggt aaa aat ggt aaa	3571
Gln Gly Pro Met Phe Tyr Lys Gln Lys Arg Tyr Gly Lys Asn Gly Lys	
1045 1050 1055	
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Ile Phe Tyr Ile Leu Lys Phe Arg Thr Met Ile Leu Asn Ala Glu Gln	
1060 1065 1070 1075	
tat cta gaa ctt aat cca gat gtt aaa gct gct tac cat gcc aac ggc	3667
Tyr Leu Glu Leu Asn Pro Asp Val Lys Ala Ala Tyr His Ala Asn Gly	
1080 1085 1090	
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Asn Lys Leu Glu Asn Asp Pro Arg Val Thr Lys Ile Gly Ser Phe Ile	
1095 1100 1105	
aga cga cac tca att gat gaa ctg cca caa ttt atc aat gtt ctt aaa	3763
Arg Arg His Ser Ile Asp Glu Leu Pro Gln Phe Ile Asn Val Leu Lys	
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Gly Asp Met Ser Leu Val Gly Pro Arg Pro Ile Leu Leu Phe Glu Ala
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 Lys Glu Tyr Gly Lys Arg Leu Ala Tyr Leu Leu Met Cys Lys Pro Gly
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 atc act ggt tat tgg acg aca cat ggt cga agt aaa gtt ctt ttt cct 3907
 Ile Thr Gly Tyr Trp Thr Thr His Gly Arg Ser Lys Val Leu Phe Pro
 1160 1165 1170
 caa cga gca gat tta gaa ctc tat tat ctc cag tac cat agc acc aaa 3955
 Gln Arg Ala Asp Leu Glu Leu Tyr Tyr Leu Gln Tyr His Ser Thr Lys
 1175 1180 1185
 aat gat atc aag ctt cta gta ctc aca att gta caa agt att aac gga 4003
 Asn Asp Ile Lys Leu Leu Val Leu Thr Ile Val Gln Ser Ile Asn Gly
 1190 1195 1200
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 Ser Asp Ala Tyr Met Lys Ile Ala Leu Val Gly Ser Ser Gly
 1205 1210 1215
 ggc cat ttg aca cac ctg tat ttg tta aaa aag ttt tgg gaa aac gaa 4099
 Gly His Leu Thr His Leu Tyr Leu Leu Lys Lys Phe Trp Glu Asn Glu
 1220 1225 1230
 gat aga ttt tgg gtc aca ttt gat aaa aca gat gca aaa tct ata ttg 4147
 Asp Arg Phe Trp Val Thr Phe Asp Lys Thr Asp Ala Lys Ser Ile Leu
 1235 1240 1245 1250
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 Lys Glu Glu Arg Phe Tyr Pro Cys Tyr Tyr Pro Thr Asn Arg Asn Val
 1255 1260 1265
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 Lys Asn Thr Ile Lys Asn Thr Ile Leu Ala Phe Lys Ile Leu Arg Lys
 1270 1275 1280
 gaa aaa cca gat ttg att att tcg agt ggt gct gcg gta gcc gtt cct 4291
 Glu Lys Pro Asp Leu Ile Ile Ser Ser Gly Ala Ala Val Ala Val Pro
 1285 1290 1295
 ttt ttt tgg tta ggt aaa cta ttc ggt gca aag aca gtc tat att gaa 4339
 Phe Phe Trp Leu Gly Lys Leu Phe Gly Ala Lys Thr Val Tyr Ile Glu
 1300 1305 1310
 ata ttt gac cgg atc gat aaa cca acc tta aca gga aaa tta gtt tat 4387
 Ile Phe Asp Arg Ile Asp Lys Pro Thr Leu Thr Gly Lys Leu Val Tyr
 1315 1320 1325 1330
 cca gtt act gat aag ttt ata gtt caa tgg gaa gag tta aaa aaa gtt 4435
 Pro Val Thr Asp Lys Phe Ile Val Gln Trp Glu Glu Leu Lys Lys Val
 1335 1340 1345
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 Tyr Pro Lys Ala Ile Asn Leu Gly Gly Ile Phe
 1350 1355
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 Gln Pro Leu Pro Glu Ser Ala Leu Lys Cys Ile Glu Ser Trp Arg Arg
 1375 1380 1385
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 Phe Cys Pro Asp Tyr Glu Ile Lys Gln Trp Ser Glu Lys Asn Tyr Asp
 1390 1395 1400
 gta aat aaa att caa tat att aag gaa gca tat caa gaa aaa aaa ttt 5156
 Val Asn Lys Ile Gln Tyr Ile Lys Glu Ala Tyr Gln Glu Lys Lys Phe
 1405 1410 1415
 gct ttt gtc acg gat gtt gca agg ctc gat ata att tgg aat gaa ggc 5204
 Ala Phe Val Thr Asp Val Ala Arg Leu Asp Ile Ile Trp Asn Glu Gly
 1420 1425 1430 1435
 ggt ata tat ctt gac acg gat gta gag ctt ata aaa tct ctt gat gaa 5252
 Gly Ile Tyr Leu Asp Thr Asp Val Glu Leu Ile Lys Ser Leu Asp Glu
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 Leu Leu Tyr Asn Ser Leu Tyr Leu Gly Met Glu Arg Ala Gly Arg Val
 1455 1460 1465
 aat acg ggt tta ggg ttt gga gct gaa gta aat cat cca att gtg aga 5348
 Asn Thr Gly Leu Gly Phe Gly Ala Glu Val Asn His Pro Ile Val Arg
 1470 1475 1480
 gct aat tta gaa ttg tat act aat att cct ttt tca ggc aat gat aat 5396
 Ala Asn Leu Glu Leu Tyr Thr Asn Ile Pro Phe Ser Gly Asn Asp Asn
 1485 1490 1495
 ata act tgt gtg acc tat acg acg aat ctt ttg aaa aaa tat ggt cta 5444
 Ile Thr Cys Val Thr Tyr Thr Asn Leu Leu Lys Lys Tyr Gly Leu
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 aaa aac aac aat gaa att caa cat ata gat aac gca ata att tta cct 5492
 Lys Asn Asn Asn Glu Ile Gln His Ile Asp Asn Ala Ile Ile Leu Pro
 1520 1525 1530
 act gaa tat tta tgt cct cta agt ttt gaa aca aat cga tta aaa ata 5540
 Thr Glu Tyr Leu Cys Pro Leu Ser Phe Glu Thr Asn Arg Leu Lys Ile
 1535 1540 1545

acg gaa aat act tac tcc atc cat cac tat gat atg agt tgg aaa gat	5588
Thr Glu Asn Thr Tyr Ser Ile His His Tyr Asp Met Ser Trp Lys Asp	
1550 1555 1560	
aag aga gat aaa ttt tta aga ctt aaa ata caa ctt aga aaa tgg gta	5636
Lys Arg Asp Lys Phe Leu Arg Leu Lys Ile Gln Leu Arg Lys Trp Val	
1565 1570 1575	
ggg gat gat ttt tat gaa aaa gtt att aaa aga att gga aaa taa ttatc	5686
Gly Asp Asp Phe Tyr Glu Lys Val Ile Lys Arg Ile Gly Lys	
1580 1585 1590	
atg aat aaa ata acc atg aca aga gag atg aga gtt att gcc tta tgt	5734
Met Asn Lys Ile Thr Met Thr Arg Glu Met Arg Val Ile Ala Leu Cys	
1595 1600 1605 1610	
gtc gta att tta gaa tat tta aat aat aca gga tta att gcg tct tca	5782
Val Val Ile Leu Glu Tyr Leu Asn Asn Thr Gly Leu Ile Ala Ser Ser	
1615 1620 1625	
gca tac tct ttt agc atg gcg agt aca atc ctc tta tcc tat atc tta	5830
Ala Tyr Ser Phe Ser Met Ala Ser Thr Ile Leu Leu Ser Tyr Ile Leu	
1630 1635 1640	
ttc tgt aaa aaa aga aaa gga ttt tct tta aag gag att att gta cta	5878
Phe Cys Lys Lys Arg Lys Gly Phe Ser Leu Lys Glu Ile Ile Val Leu	
1645 1650 1655	
cta att cca ttt att ttt gta gtt tta aat cgt gat cct agt aat ttc	5926
Leu Ile Pro Phe Ile Phe Val Val Leu Asn Arg Asp Pro Ser Asn Phe	
1660 1665 1670	
agt tta ggg tta atg tgg ata ctc tat ttt atg tta agt aag tcg gaa	5974
Ser Leu Gly Leu Met Trp Ile Leu Tyr Phe Met Leu Ser Lys Ser Glu	
1675 1680 1685 1690	
ata gat tta aaa aaa gtg atg aaa aca ttt ttt gtt acc tct agt gtt	6022
Ile Asp Leu Lys Lys Val Met Lys Thr Phe Phe Val Thr Ser Ser Val	
1695 1700 1705	
tgt ttt att ttg aca ata gta ctt tat tta ata atg tct ctt aat aaa	6070
Cys Phe Ile Leu Thr Ile Val Leu Tyr Leu Ile Met Ser Leu Asn Lys	
1710 1715 1720	
agc tct gat atg ata atg tgg cgt gga gat gct ttt ata aat cgt atg	6118
Ser Ser Asp Met Ile Met Trp Arg Gly Asp Ala Phe Ile Asn Arg Met	
1725 1730 1735	
agt tta gga ttt atc caa ccg aat ttt gca atg atg agc ttt tta ggt	6166
Ser Leu Gly Phe Ile Gln Pro Asn Phe Ala Met Met Ser Phe Leu Gly	
1740 1745 1750	
ata gcg ata gcc tta tta tat ttg agt act gaa aga caa aga ata act	6214
Ile Ala Ile Ala Leu Leu Tyr Leu Ser Thr Glu Arg Gln Arg Ile Thr	
1755 1760 1765 1770	
ata att ttt att gcc att gta act ttt att ata ttt tac ttt act caa	6262
Ile Ile Phe Ile Ala Ile Val Thr Phe Ile Ile Phe Tyr Phe Thr Gln	
1775 1780 1785	

tca aga act tca gga tat atc tta ttt ttt att ttg agt att tta ttt 6310
 Ser Arg Thr Ser Gly Tyr Ile Leu Phe Phe Ile Leu Ser Ile Leu Phe
 1790 1795 1800

gtt agt agt aaa aaa act aaa aag caa gtt tca aat ttt gaa aaa agg 6358
 Val Ser Ser Lys Lys Thr Lys Lys Gln Val Ser Asn Phe Glu Lys Arg
 1805 1810 1815

agc att aca gtt tta cca cta ctt ctt tta atc atc tct tat tcg ttg 6406
 Ser Ile Thr Val Leu Pro Leu Leu Leu Leu Ile Ile Ser Tyr Ser Leu
 1820 1825 1830

tta aag tta cct att aat caa tac atc aat agc ttg ctt tct ggt cgt 6454
 Leu Lys Leu Pro Ile Asn Gln Tyr Ile Asn Ser Leu Leu Ser Gly Arg
 1835 1840 1845 1850

ctg gcg ctt tat caa gag att tat tct aca ttt ggt ata cat ttg ata 6502
 Leu Ala Leu Tyr Gln Glu Ile Tyr Ser Thr Phe Gly Ile His Leu Ile
 1855 1860 1865

ggg aat aat gat gtt aaa aat aca atg tta gat aca gca tat ctt caa 6550
 Gly Asn Asn Asp Val Lys Asn Thr Met Leu Asp Thr Ala Tyr Leu Gln
 1870 1875 1880

agt ttg cta gca aaa gga att ttg ttt aca ttg ttt tta ttt gta act 6598
 Ser Leu Leu Ala Lys Gly Ile Leu Phe Thr Leu Phe Leu Phe Val Thr
 1885 1890 1895

ttc ttt ttc ata ttt ttt ctt aag aga aaa aca caa act agg ttg caa 6646
 Phe Phe Phe Ile Phe Phe Leu Lys Arg Lys Thr Gln Thr Arg Leu Gln
 1900 1905 1910

agt tta gta att atg atg tat ttt tta att gca ttt aca gaa aca tca 6694
 Ser Leu Val Ile Met Met Tyr Phe Leu Ile Ala Phe Thr Glu Thr Ser
 1915 1920 1925 1930

ttt ttt agg ttt gta att tta ttt cca gta ttg atg gta ata atg gat 6742
 Phe Phe Arg Phe Val Ile Leu Phe Pro Val Leu Met Val Ile Met Asp
 1935 1940 1945

cag aaa gag gct aat aaa gta ata gaa aag gtg gca tag tgagtattaa 6791
 Gln Lys Glu Ala Asn Lys Val Ile Glu Lys Val Ala
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<212> PRT

<213> Lactococcus lactis

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 20 25 30
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 35 40 45
 Ile Gly Leu Ser Glu Tyr Phe Asn Val Ser Pro Lys Tyr Leu Met Gly
 50 55 60

Ile Ile Asp Glu Pro Asn Asp Ser Ser Ala Ile Asn Leu Phe Lys Thr
 65 70 75 80
 Leu Thr Gln Glu Glu Lys Lys Glu Met Phe Ile Ile Cys Gln Lys Trp
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 Leu Phe Leu Glu Tyr Gln Ile Glu Leu
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 Pro Asp Val Val Leu Tyr Glu Ala Pro Leu Phe Asn Asp Asn Gln Asn
 35 40 45
 Ile Glu Ala Thr Ala Ser Trp Thr Ser Asn Glu Gln Leu Ile Thr Asn
 50 55 60
 Leu Ala Ser Thr Gly Ala Glu Val Ile Val Gln Pro Ser Pro Pro Ile
 65 70 75 80
 Tyr Gly Gly Val Val Tyr Pro Val Gln Glu Gln Phe Lys Gln Ser
 85 90 95
 Leu Ser Thr Lys Tyr Pro Tyr Ile Asp Tyr Trp Ala Ser Tyr Pro Asp
 100 105 110
 Lys Asn Ser Asp Glu Met Lys Gly Leu Val Ser Asp Asp Gly Val Tyr
 115 120 125
 Arg Thr Leu Asn Ala Ser Gly Asn Lys Val Trp Leu Asp Tyr Ile Thr
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 Lys Tyr Phe Thr Ala Asn
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 Thr Ile Leu Gly Ser Ile Tyr Thr Phe Phe Ile Ala Ser Pro Val Tyr
 35 40 45
 Thr Ala Ser Thr Gln Leu Val Val Lys Leu Pro Asn Ser Glu His Ser
 50 55 60
 Ala Ala Tyr Ala Gly Glu Val Thr Gly Asn Ile Gln Met Ala Asn Thr
 65 70 75 80
 Ile Asn Gln Val Ile Val Ser Pro Val Ile Leu Asp Lys Val Gln Ser
 85 90 95
 Asn Leu Asn Leu Ser Asp Gly Ser Phe Gln Lys Gln Val Thr Val Ala
 100 105 110
 Asn Gln Thr Asp Ser Gln Val Ile Thr Leu Thr Val Lys Tyr Ser Asn
 115 120 125
 Pro Tyr Ile Ala Gln Lys Ile Ala Asp Glu Thr Ala Lys Ile Phe Ser
 130 135 140
 Ser Asp Ala Ala Lys Leu Leu Asn Val Thr Asn Val Asn Ile Leu Ser

145 150 155 160
 Lys Ala Lys Ala Gln Thr Thr Pro Ile Ser Pro Lys Pro Lys Leu Tyr
 165 170 175
 Leu Ala Ile Ser Val Ile Ala Gly Leu Val Leu Gly Leu Ala Ile Ala
 180 185 190
 Leu Leu Lys Glu Leu Phe Asp Asn Lys Ile Asn Lys Glu Glu Asp Ile
 195 200 205
 Glu Ala Leu Gly Leu Thr Val Leu Gly Val Thr Ser Tyr Ala Gln Met
 210 215 220
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 Asn Lys Arg

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 35 40 45
 Val Ala Ser Ser Glu Val Ala Val Gly Lys Ser Thr Val Cys Ala Asn
 50 55 60
 Ile Ala Val Ala Phe Ala Gln Gln Gly Lys Lys Val Leu Leu Ile Asp
 65 70 75 80
 Gly Asp Leu Arg Lys Pro Thr Val Asn Ile Thr Phe Lys Val Gln Asn
 85 90 95
 Arg Val Gly Leu Thr Asn Ile Leu Met His Gln Ser Ser Ile Glu Asp
 100 105 110
 Ala Ile Gln Gly Thr Arg Leu Ser Glu Asn Leu Thr Ile Ile Thr Ser
 115 120 125
 Gly Pro Ile Pro Pro Asn Pro Ser Glu Leu Leu Ala Ser Ser Ala Met
 130 135 140
 Lys Asn Leu Ile Asp Ser Val Ser Asp Leu Phe Asp Val Val Leu Ile
 145 150 155 160
 Asp Thr Pro Thr Leu Ser Ala Val Thr Asp Ala Gln Ile Leu Ser Ser
 165 170 175
 Tyr Val Gly Gly Ala Val Ile Val Val Arg Ala Tyr Glu Thr Lys Lys
 180 185 190
 Glu Ser Leu Ala Lys Thr Lys Lys Met Leu Glu Gln Val Asn Thr Asn
 195 200 205
 Ile Leu Gly Val Val Leu His Gly Val Asn Ser Ser Glu Ser Pro Ser
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 Tyr Tyr Tyr His Gly Val Glu
 225 230

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Lys	Val	Lys	Glu	Val	Gln	Asn	Ile	Ile	Asp	Glu	His	Gln	Leu	Pro	Ile		
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Glu	Val	Leu	Pro	Gly	Gln	Glu	Val	Arg	Ile	Tyr	Gly	Asp	Leu	Leu	Lys		
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Glu	Phe	Ser	Glu	Gly	Lys	Leu	Leu	Thr	Ala	Ala	Gly	Thr	Ser	Ser	Tyr		
	65				70					75					80		
Ile	Leu	Ile	Glu	Phe	Pro	Ser	Asn	His	Val	Pro	Ala	Tyr	Ala	Lys	Glu		
			85					90						95			
Leu	Phe	Tyr	Asn	Ile	Gln	Leu	Glu	Gly	Leu	Gln	Pro	Ile	Leu	Val	His		
			100					105					110				
Pro	Glu	Arg	Asn	Ser	Gly	Ile	Ile	Glu	Asn	Pro	Asp	Ile	Leu	Phe	Asp		
	115					120						125					
Phe	Ile	Glu	Gln	Gly	Val	Leu	Ser	Gln	Ile	Thr	Ala	Ser	Ser	Val	Thr		
	130				135						140						
Gly	His	Phe	Gly	Lys	Lys	Ile	Gln	Lys	Leu	Ser	Phe	Lys	Met	Ile	Glu		
	145			150					155					160			
Asn	His	Leu	Thr	His	Phe	Val	Ala	Ser	Asp	Ala	His	Asn	Val	Thr	Ser		
			165					170						175			
Arg	Ala	Phe	Lys	Met	Lys	Glu	Ala	Phe	Glu	Ile	Ile	Glu	Asp	Ser	Tyr		
		180						185					190				
Gly	Ser	Asp	Val	Ser	Arg	Met	Phe	Gln	Asn	Asn	Ala	Glu	Ser	Val	Ile		
	195					200					205						
Leu	Asn	Glu	Ser	Phe	Tyr	Gln	Glu	Lys	Pro	Thr	Lys	Ile	Lys	Thr	Lys		
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Lys	Phe	Leu	Gly	Leu	Phe												
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<211> 228

<212> PRT

<213> Lactococcus lactis

<400> 7

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		20						25				30					
Ala	Ile	Asp	Ile	Leu	Gly	Gly	Leu	Ala	Gly	Ser	Val	Leu	Phe	Leu	Ile		
		35				40						45					
Ala	Ala	Ala	Leu	Leu	Tyr	Ile	Pro	Tyr	Lys	Met	Ser	Ser	Lys	Lys	Asp		
	50				55					60							
Gln	Gly	Pro	Met	Phe	Tyr	Lys	Gln	Lys	Arg	Tyr	Gly	Lys	Asn	Gly	Lys		
	65				70				75					80			
Ile	Phe	Tyr	Ile	Leu	Lys	Phe	Arg	Thr	Met	Ile	Leu	Asn	Ala	Glu	Gln		
			85					90						95			
Tyr	Leu	Glu	Leu	Asn	Pro	Asp	Val	Lys	Ala	Ala	Tyr	His	Ala	Asn	Gly		
		100						105				110					
Asn	Lys	Leu	Glu	Asn	Asp	Pro	Arg	Val	Thr	Lys	Ile	Gly	Ser	Phe	Ile		
	115					120						125					
Arg	Arg	His	Ser	Ile	Asp	Glu	Leu	Pro	Gln	Phe	Ile	Asn	Val	Leu	Lys		
	130					135					140						
Gly	Asp	Met	Ser	Leu	Val	Gly	Pro	Arg	Pro	Ile	Leu	Leu	Phe	Glu	Ala		
	145				150				155					160			
Lys	Glu	Tyr	Gly	Lys	Arg	Leu	Ala	Tyr	Leu	Leu	Met	Cys	Lys	Pro	Gly		
			165					170						175			
Ile	Thr	Gly	Tyr	Trp	Thr	Thr	His	Gly	Arg	Ser	Lys	Val	Leu	Phe	Pro		
		180						185					190				

Gln Arg Ala Asp Leu Glu Leu Tyr Tyr Leu Gln Tyr His Ser Thr Lys
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 Asn Asp Ile Lys Leu Leu Val Leu Thr Ile Val Gln Ser Ile Asn Gly
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 Ser Asp Ala Tyr
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 Phe Asp Lys Thr Asp Ala Lys Ser Ile Leu Lys Glu Glu Arg Phe Tyr
 35 40 45
 Pro Cys Tyr Tyr Pro Thr Asn Arg Asn Val Lys Asn Thr Ile Lys Asn
 50 55 60
 Thr Ile Leu Ala Phe Lys Ile Leu Arg Lys Glu Lys Pro Asp Leu Ile
 65 70 75 80
 Ile Ser Ser Gly Ala Val Ala Val Pro Phe Phe Trp Leu Gly Lys
 85 90 95
 Leu Phe Gly Ala Lys Thr Val Tyr Ile Glu Ile Phe Asp Arg Ile Asp
 100 105 110
 Lys Pro Thr Leu Thr Gly Lys Leu Val Tyr Pro Val Thr Asp Lys Phe
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 Leu Gly Gly Ile Phe
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 35 40 45
 Ile Gln Tyr Ile Lys Glu Ala Tyr Gln Glu Lys Lys Phe Ala Phe Val
 50 55 60
 Thr Asp Val Ala Arg Leu Asp Ile Ile Trp Asn Glu Gly Gly Ile Tyr
 65 70 75 80
 Leu Asp Thr Asp Val Glu Leu Ile Lys Ser Leu Asp Glu Leu Leu Tyr
 85 90 95
 Asn Ser Leu Tyr Leu Gly Met Glu Arg Ala Gly Arg Val Asn Thr Gly
 100 105 110
 Leu Gly Phe Gly Ala Glu Val Asn His Pro Ile Val Arg Ala Asn Leu
 115 120 125
 Glu Leu Tyr Thr Asn Ile Pro Phe Ser Gly Asn Asp Asn Ile Thr Cys
 130 135 140
 Val Thr Tyr Thr Thr Asn Leu Leu Lys Lys Tyr Gly Leu Lys Asn Asn

145					150					155				160
Asn	Glu	Ile	Gln	His	Ile	Asp	Asn	Ala	Ile	Ile	Leu	Pro	Thr	Glu Tyr
				165					170					175
Leu	Cys	Pro	Leu	Ser	Phe	Glu	Thr	Asn	Arg	Leu	Lys	Ile	Thr	Glu Asn
			180					185					190	
Thr	Tyr	Ser	Ile	His	His	Tyr	Asp	Met	Ser	Trp	Lys	Asp	Lys	Arg Asp
		195					200					205		
Lys	Phe	Leu	Arg	Leu	Lys	Ile	Gln	Leu	Arg	Lys	Trp	Val	Gly	Asp Asp
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<212> PRT

<213> Lactococcus lactis

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Ala	Tyr	Ser	Phe	Ser	Met	Ala	Ser	Thr	Ile	Leu	Leu	Ser	Tyr	Ile	Leu
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Phe	Cys	Lys	Lys	Arg	Lys	Gly	Phe	Ser	Leu	Lys	Glu	Ile	Ile	Val	Leu
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Leu	Ile	Pro	Phe	Ile	Phe	Val	Val	Leu	Asn	Arg	Asp	Pro	Ser	Asn	Phe
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Ser	Leu	Gly	Leu	Met	Trp	Ile	Leu	Tyr	Phe	Met	Leu	Ser	Lys	Ser	Glu
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Ile	Asp	Leu	Lys	Lys	Val	Met	Lys	Thr	Phe	Phe	Val	Thr	Ser	Ser	Val
			100					105					110		
Cys	Phe	Ile	Leu	Thr	Ile	Val	Leu	Tyr	Leu	Ile	Met	Ser	Leu	Asn	Lys
		115					120					125			
Ser	Ser	Asp	Met	Ile	Met	Trp	Arg	Gly	Asp	Ala	Phe	Ile	Asn	Arg	Met
	130					135					140				
Ser	Leu	Gly	Phe	Ile	Gln	Pro	Asn	Phe	Ala	Met	Met	Ser	Phe	Leu	Gly
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Ile	Ala	Ile	Ala	Leu	Leu	Tyr	Leu	Ser	Thr	Glu	Arg	Gln	Arg	Ile	Thr
			165						170					175	
Ile	Ile	Phe	Ile	Ala	Ile	Val	Thr	Phe	Ile	Ile	Phe	Tyr	Phe	Thr	Gln
			180					185					190		
Ser	Arg	Thr	Ser	Gly	Tyr	Ile	Leu	Phe	Phe	Ile	Leu	Ser	Ile	Leu	Phe
		195					200					205			
Val	Ser	Ser	Lys	Lys	Thr	Lys	Lys	Gln	Val	Ser	Asn	Phe	Glu	Lys	Arg
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Ser	Ile	Thr	Val	Leu	Pro	Leu	Leu	Leu	Leu	Ile	Ile	Ser	Tyr	Ser	Leu
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Leu	Lys	Leu	Pro	Ile	Asn	Gln	Tyr	Ile	Asn	Ser	Leu	Leu	Ser	Gly	Arg
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Leu	Ala	Leu	Tyr	Gln	Glu	Ile	Tyr	Ser	Thr	Phe	Gly	Ile	His	Leu	Ile
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Gly	Asn	Asn	Asp	Val	Lys	Asn	Thr	Met	Leu	Asp	Thr	Ala	Tyr	Leu	Gln
		275					280					285			
Ser	Leu	Leu	Ala	Lys	Gly	Ile	Leu	Phe	Thr	Leu	Phe	Leu	Phe	Val	Thr
	290					295					300				
Phe	Phe	Phe	Ile	Phe	Phe	Leu	Lys	Arg	Lys	Thr	Gln	Thr	Arg	Leu	Gln
305					310					315					320
Ser	Leu	Val	Ile	Met	Met	Tyr	Phe	Leu	Ile	Ala	Phe	Thr	Glu	Thr	Ser
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 35 40 45
 Trp Glu Lys Phe Ile Gly Tyr Glu Thr Met Glu Arg Cys Met Asn Glu
 50 55 60
 Ala Ser Thr Ile Ile Thr His Gly Gly Pro Ser Thr Tyr Met Gln Val
 65 70 75 80
 Leu Gln Leu Gly Lys Ile Pro Ile Val Val Pro Arg Gln Met Lys Phe
 85 90 95
 Asp Glu His Ile Asn Asp His Gln Leu Trp Val Ser Lys Gln Val Val
 100 105 110
 Lys Lys Gly Tyr Ser Leu Ile Leu Cys Glu Asp Val Glu Asp Ile Leu
 115 120 125
 Glu Asn Ile Ile Ser Ser Lys Ile Ser Asp Thr Leu Gln Lys Asn Val
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 Leu Ser Glu Asn Leu Ile Ser Ile Ile Val Pro Val Tyr Asn Ser Glu
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 aag tat tta aga gcg gct att cat agt cta tta aat caa act tat caa 156
 Lys Tyr Leu Arg Ala Ala Ile His Ser Leu Leu Asn Gln Thr Tyr Gln
 20 25 30

aat att gaa gtt att ttg att aat gat ggg tcc act gat ggc tca caa	204
Asn Ile Glu Val Ile Leu Ile Asn Asp Gly Ser Thr Asp Gly Ser Gln	
35 40 45	
gag cta att agc tca ttt caa aaa aag gat aaa aga att aaa tta tat	252
Glu Leu Ile Ser Ser Phe Gln Lys Lys Asp Lys Arg Ile Lys Leu Tyr	
50 55 60	
aat act aaa aat ctg ggg gta tcg cat gcg aga aat tat ggt att gat	300
Asn Thr Lys Asn Leu Gly Val Ser His Ala Arg Asn Tyr Gly Ile Asp	
65 70 75 80	
aga gct agt ggt tcg tat att atg ttt tta gac cca gac gac act tat	348
Arg Ala Ser Gly Ser Tyr Ile Met Phe Leu Asp Pro Asp Asp Thr Tyr	
85 90 95	
gat aaa agt tac tgt tta gaa atg att ggg ttg att aat aag ttt aat	396
Asp Lys Ser Tyr Cys Leu Glu Met Ile Gly Leu Ile Asn Lys Phe Asn	
100 105 110	
gct gat gtt gtt atg agt aat tac tat ata tgc aaa ggc aaa aat ata	444
Ala Asp Val Val Met Ser Asn Tyr Tyr Ile Cys Lys Gly Lys Asn Ile	
115 120 125	
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Tyr Pro Asn Val Asn Asn Asp Leu Leu Glu Cys Glu Gly Leu Leu Ser	
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agg gat aaa aca atg cgt tca ata cta tct gat aca ggt ttt aaa ggg	540
Arg Asp Lys Thr Met Arg Ser Ile Leu Ser Asp Thr Gly Phe Lys Gly	
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Phe Val Trp Thr Arg Ile Phe Arg Lys Asn Val Ile Asn Asn Val Lys	
165 170 175	
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Phe Asn Glu Ser Ile Asn Tyr Leu Glu Asp Met Leu Phe Asn Ile Ser	
180 185 190	
att gta cat aat gca aga att ata gcc tat aca aat aaa aga cat tat	684
Ile Val His Asn Ala Arg Ile Ile Ala Tyr Thr Asn Lys Arg His Tyr	
195 200 205	
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Phe Tyr Leu Gln Arg Glu Asp Ser Ala Ser Lys Lys Phe Ser Lys Ser	
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Phe Phe Lys Ser Leu Asn Leu Ile Arg Gly Lys Val Asp Pro Glu Phe	
225 230 235 240	
tat tcg caa att gat tct gtt att ttt tat aat tta gtt gga tgg tta	828
Tyr Ser Gln Ile Asp Ser Val Ile Phe Tyr Asn Leu Val Gly Trp Leu	
245 250 255	
ata act gag aga aag agt agg gaa aat agt caa ttt ata agg aga aat	876
Ile Thr Glu Arg Lys Ser Arg Glu Asn Ser Gln Phe Ile Arg Arg Asn	
260 265 270	
att aaa aat atg aaa tcc caa gtt aag ttt aaa acg ctt aaa atg gaa	924

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Asn	Pro	Ile	Lys	Asn	Leu	Ile	Leu	Lys	Leu	Ser	Tyr	Ala	Phe	Pro	Leu		
	290					295				300							
gta	gga	tcg	tgt	atg	ata	cat	atg	tta	tcc	gtt	ttt	atg	aaa	acc	aaa	1020	
Val	Gly	Ser	Cys	Met	Ile	His	Met	Leu	Ser	Val	Phe	Met	Lys	Thr	Lys		
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Leu	Tyr	Ser	Lys	Leu	Met	Ser	Met	Leu	Arg	Lys	Gly						
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			Met	Asn	Pro	Leu	Ile	Ser	Ile	Ile						340	
						335											
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cta	ttg	aaa	caa	acg	aac	aag	aat	ttt	gag	gtt	att	ttt	att	gat	gac	1455	
Leu	Leu	Lys	Gln	Thr	Asn	Lys	Asn	Phe	Glu	Val	Ile	Phe	Ile	Asp	Asp		
			360					365					370				
gga	tca	act	gat	gaa	agc	atg	caa	att	ttg	aaa	gaa	ata	atg	gca	ggc	1503	
Gly	Ser	Thr	Asp	Glu	Ser	Met	Gln	Ile	Leu	Lys	Glu	Ile	Met	Ala	Gly		
		375					380					385					
agt	gaa	caa	gaa	ttt	tcg	ttc	aag	ttg	ttg	caa	caa	gtt	aat	cag	ggg	1551	
Ser	Glu	Gln	Glu	Phe	Ser	Phe	Lys	Leu	Leu	Gln	Gln	Val	Asn	Gln	Gly		
	390					395					400						
tta	tct	tca	gcc	agg	aat	atc	ggg	ata	ctt	aat	gca	act	gga	gaa	tat	1599	
Leu	Ser	Ser	Ala	Arg	Asn	Ile	Gly	Ile	Leu	Asn	Ala	Thr	Gly	Glu	Tyr		
405					410				415						420		
atc	ttt	ttt	ttg	gat	tca	gat	gat	gaa	ata	gaa	agc	aat	ttt	gtg	gag	1647	
Ile	Phe	Phe	Leu	Asp	Ser	Asp	Asp	Glu	Ile	Glu	Ser	Asn	Phe	Val	Glu		
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aca	att	ttg	act	agt	tgc	tat	aaa	tac	agt	caa	ccg	gat	aca	ctt	atc	1695	
Thr	Ile	Leu	Thr	Ser	Cys	Tyr	Lys	Tyr	Ser	Gln	Pro	Asp	Thr	Leu	Ile		
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Phe	Asp	Tyr	Ser	Ser	Ile	Asp	Glu	Phe	Gly	Asn	Ala	Leu	Asp	Ser	Asn		
		455					460					465					
tat	ggg	cat	gga	agt	att	tat	cgt	caa	aaa	gat	ttg	tgt	aca	agt	gag	1791	
Tyr	Gly	His	Gly	Ser	Ile	Tyr	Arg	Gln	Lys	Asp	Leu	Cys	Thr	Ser	Glu		

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caa ata tta act gca ttg tct aaa gat gag ata cca aca act gca tgg			1839
Gln Ile Leu Thr Ala Leu Ser Lys Asp Glu Ile Pro Thr Thr Ala Trp			
485	490	495	500
tca ttt gta aca aaa cgc tct gtg att gaa aaa cac gat tta cta ttt			1887
Ser Phe Val Thr Lys Arg Ser Val Ile Glu Lys His Asp Leu Leu Phe			
	505	510	515
tct gtt gga aaa aaa ttt gaa gat aac aat ttt acg ccg aaa gtt ttt			1935
Ser Val Gly Lys Lys Phe Glu Asp Asn Asn Phe Thr Pro Lys Val Phe			
	520	525	530
tac ttt agt aaa aac att gtt gtt att tcc cta aga ttg tat aga tat			1983
Tyr Phe Ser Lys Asn Ile Val Val Ile Ser Leu Arg Leu Tyr Arg Tyr			
	535	540	545
agg aaa cgc tct ggg tct att atg agt aat cgc ccg gaa aaa ttc ttt			2031
Arg Lys Arg Ser Gly Ser Ile Met Ser Asn Arg Pro Glu Lys Phe Phe			
	550	555	560
tcg gac gac gcc att ttt gta aca tat gac tta tta gat ttt tat gat			2079
Ser Asp Asp Ala Ile Phe Val Thr Tyr Asp Leu Leu Asp Phe Tyr Asp			
	565	570	580
cag tat aaa att cgg gaa ttg gga gca gta gtt ggt aaa ata gtt atg			2127
Gln Tyr Lys Ile Arg Glu Leu Gly Ala Val Val Gly Lys Ile Val Met			
	585	590	595
aca aca tta gct tct ttt cca gat tcg aaa aaa ttg tat aat gaa tta			2175
Thr Thr Leu Ala Ser Phe Pro Asp Ser Lys Lys Leu Tyr Asn Glu Leu			
	600	605	610
aat cca atc aga aaa aaa gta ttt aaa gat tat att tca ata gaa aaa			2223
Asn Pro Ile Arg Lys Lys Val Phe Lys Asp Tyr Ile Ser Ile Glu Lys			
	615	620	625
aga cat act aaa cgg ata aaa atg tat gta aaa atg tat gtt ttt tct			2271
Arg His Thr Lys Arg Ile Lys Met Tyr Val Lys Met Tyr Val Phe Ser			
	630	635	640
tct tat gtt gga tat aaa ctt tac aga ctg gta aaa ggt aaa cac tgg			2319
Ser Tyr Val Gly Tyr Lys Leu Tyr Arg Leu Val Lys Gly Lys His Trp			
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Lys			

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 Asn Ile Glu Val Ile Leu Ile Asn Asp Gly Ser Thr Asp Gly Ser Gln
 35 40 45
 Glu Leu Ile Ser Ser Phe Gln Lys Lys Asp Lys Arg Ile Lys Leu Tyr
 50 55 60
 Asn Thr Lys Asn Leu Gly Val Ser His Ala Arg Asn Tyr Gly Ile Asp
 65 70 75 80
 Arg Ala Ser Gly Ser Tyr Ile Met Phe Leu Asp Pro Asp Asp Thr Tyr
 85 90 95
 Asp Lys Ser Tyr Cys Leu Glu Met Ile Gly Leu Ile Asn Lys Phe Asn
 100 105 110
 Ala Asp Val Val Met Ser Asn Tyr Tyr Ile Cys Lys Gly Lys Asn Ile
 115 120 125
 Tyr Pro Asn Val Asn Asn Asp Leu Leu Glu Cys Glu Gly Leu Leu Ser
 130 135 140
 Arg Asp Lys Thr Met Arg Ser Ile Leu Ser Asp Thr Gly Phe Lys Gly
 145 150 155 160
 Phe Val Trp Thr Arg Ile Phe Arg Lys Asn Val Ile Asn Asn Val Lys
 165 170 175
 Phe Asn Glu Ser Ile Asn Tyr Leu Glu Asp Met Leu Phe Asn Ile Ser
 180 185 190
 Ile Val His Asn Ala Arg Ile Ile Ala Tyr Thr Asn Lys Arg His Tyr
 195 200 205
 Phe Tyr Leu Gln Arg Glu Asp Ser Ala Ser Lys Lys Phe Ser Lys Ser
 210 215 220
 Phe Phe Lys Ser Leu Asn Leu Ile Arg Gly Lys Val Asp Pro Glu Phe
 225 230 235 240
 Tyr Ser Gln Ile Asp Ser Val Ile Phe Tyr Asn Leu Val Gly Trp Leu
 245 250 255
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 260 265 270
 Ile Lys Asn Met Lys Ser Gln Val Lys Phe Lys Thr Leu Lys Met Glu
 275 280 285
 Asn Pro Ile Lys Asn Leu Ile Leu Lys Leu Ser Tyr Ala Phe Pro Leu
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Phe Glu Val Ile Phe Ile Asp Asp Gly Ser Thr Asp Glu Ser Met Gln
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Ile Leu Lys Glu Ile Met Ala Gly Ser Glu Gln Glu Phe Ser Phe Lys
           50           55           60

Leu Leu Gln Gln Val Asn Gln Gly Leu Ser Ser Ala Arg Asn Ile Gly
65           70           75           80

Ile Leu Asn Ala Thr Gly Glu Tyr Ile Phe Phe Leu Asp Ser Asp Asp
           85           90           95

Glu Ile Glu Ser Asn Phe Val Glu Thr Ile Leu Thr Ser Cys Tyr Lys
           100          105          110

Tyr Ser Gln Pro Asp Thr Leu Ile Phe Asp Tyr Ser Ser Ile Asp Glu
           115          120          125

Phe Gly Asn Ala Leu Asp Ser Asn Tyr Gly His Gly Ser Ile Tyr Arg
           130          135          140

Gln Lys Asp Leu Cys Thr Ser Glu Gln Ile Leu Thr Ala Leu Ser Lys
145          150          155          160

Asp Glu Ile Pro Thr Thr Ala Trp Ser Phe Val Thr Lys Arg Ser Val
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Ile Glu Lys His Asp Leu Leu Phe Ser Val Gly Lys Lys Phe Glu Asp
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Asn Asn Phe Thr Pro Lys Val Phe Tyr Phe Ser Lys Asn Ile Val Val
           195          200          205

Ile Ser Leu Arg Leu Tyr Arg Tyr Arg Lys Arg Ser Gly Ser Ile Met
           210          215          220

Ser Asn Arg Pro Glu Lys Phe Phe Ser Asp Asp Ala Ile Phe Val Thr
225          230          235          240

Tyr Asp Leu Leu Asp Phe Tyr Asp Gln Tyr Lys Ile Arg Glu Leu Gly
           245          250          255

Ala Val Val Gly Lys Ile Val Met Thr Thr Leu Ala Ser Phe Pro Asp
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Ser Lys Lys Leu Tyr Asn Glu Leu Asn Pro Ile Arg Lys Lys Val Phe
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Arg Leu Val Lys Gly Lys His Trp Lys
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 Tyr Gln Val Phe Ile Ile Ile Val Pro Leu Leu Thr Ile Pro Tyr Leu
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 Ser Arg Ile Leu Gly Pro Ser Gly Ile Gly Ile Asn Ser Tyr Thr Asn
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 Ser Ile Val Gln Tyr Phe Val Leu Phe Gly Ser Ile Gly Val Gly Leu
 45 50 55 60

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 Tyr Gly Asn Arg Gln Ile Ala Phe Val Arg Asp Asn Gln Val Lys Met
 65 70 75

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 Ser Lys Val Phe Tyr Glu Ile Phe Ile Leu Arg Leu Phe Thr Ile Cys
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 Leu Ala Tyr Phe Leu Phe Val Ala Phe Leu Ile Ile Asn Gly Gln Tyr
 95 100 105

cat gca tac tat ttg tct caa tcc att gct ata gtt gca gct gca ttt 447
 His Ala Tyr Tyr Leu Ser Gln Ser Ile Ala Ile Val Ala Ala Ala Phe
 110 115 120

gat atc tct tgg ttt ttt atg gga att gaa aat ttt aaa gta act gta 495
 Asp Ile Ser Trp Phe Phe Met Gly Ile Glu Asn Phe Lys Val Thr Val
 125 130 135 140

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 145 150 155

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Phe Val Lys Ser Tyr Asn Asp Leu Asn Ile Tyr Ile Leu Ile Thr Val	
160 165 170	
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Leu Ser Thr Leu Ile Gly Asn Leu Thr Phe Phe Pro Ser Leu His Arg	
175 180 185	
tat ctg gta aag gtt aac tat cgt gaa tta agg cca ata aag cat tta	687
Tyr Leu Val Lys Val Asn Tyr Arg Glu Leu Arg Pro Ile Lys His Leu	
190 195 200	
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Lys Gln Ser Leu Val Met Phe Ile Pro Gln Ile Ala Val Gln Ile Tyr	
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Trp Val Leu Asn Lys Thr Met Leu Gly Ser Leu Asp Ser Val Thr Ser	
225 230 235	
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Ser Gly Phe Phe Asp Gln Ser Asp Lys Ile Val Lys Leu Val Leu Ala	
240 245 250	
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Ile Ala Thr Ala Thr Gly Thr Val Met Leu Pro Arg Val Ala Asn Ala	
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Phe Ala His Arg Glu Tyr Ser Lys Ile Lys Glu Tyr Met Tyr Ala Gly	
270 275 280	
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Phe Ser Phe Val Ser Ala Ile Ser Ile Pro Met Met Phe Gly Leu Ile	
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Ala Ile Thr Pro Lys Phe Val Pro Leu Phe Phe Thr Ser Gln Phe Ser	
305 310 315	
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Asp Val Ile Pro Val Leu Met Ile Glu Ser Ile Ala Ile Ile Phe Ile	
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Ala Trp Ser Asn Ala Ile Gly Thr Gln Tyr Leu Leu Pro Thr Asn Gln	
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Asn Lys Ser Tyr Thr Val Ser Val Ile Ile Gly Ala Ile Val Asn Leu	
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Met Leu Asn Ile Pro Leu Ile Ile Tyr Leu Gly Thr Val Gly Ala Ser	
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Ile Ala Thr Val Ile Ser Glu Met Ser Val Thr Val Tyr Gln Leu Phe	
385 390 395	

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 Ile Ile His Lys Gln Leu Asn Leu His Thr Leu Phe Ala Asp Leu Ser
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 Lys Tyr Leu Ile Ala Gly Leu Val Met Phe Leu Ile Val Phe Lys Ile
 415 420 425
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 Ser Leu Leu Thr Pro Thr Ser Trp Ile Phe Ile Leu Leu Glu Ile Thr
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 Val Gly Ile Ile Ile Tyr Val Val Leu Leu Ile Phe Leu Lys Ala Glu
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 Ile Ile Asn Lys Leu Lys Phe Ile Met His Lys
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<212> PRT

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 Gly Pro Ser Gly Ile Gly Ile Asn Ser Tyr Thr Asn Ser Ile Val Gln
 35 40 45
 Tyr Phe Val Leu Phe Gly Ser Ile Gly Val Gly Leu Tyr Gly Asn Arg
 50 55 60
 Gln Ile Ala Phe Val Arg Asp Asn Gln Val Lys Met Ser Lys Val Phe
 65 70 75 80
 Tyr Glu Ile Phe Ile Leu Arg Leu Phe Thr Ile Cys Leu Ala Tyr Phe
 85 90 95
 Leu Phe Val Ala Phe Leu Ile Ile Asn Gly Gln Tyr His Ala Tyr Tyr
 100 105 110
 Leu Ser Gln Ser Ile Ala Ile Val Ala Ala Ala Phe Asp Ile Ser Trp
 115 120 125
 Phe Phe Met Gly Ile Glu Asn Phe Lys Val Thr Val Leu Arg Asn Phe
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 Ile Val Lys Leu Leu Ala Leu Phe Ser Ile Phe Leu Phe Val Lys Ser
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Tyr Asn Asp Leu Asn Ile Tyr Ile Leu Ile Thr Val Leu Ser Thr Leu
 165 170 175
 Ile Gly Asn Leu Thr Phe Phe Pro Ser Leu His Arg Tyr Leu Val Lys
 180 185 190
 Val Asn Tyr Arg Glu Leu Arg Pro Ile Lys His Leu Lys Gln Ser Leu
 195 200 205
 Val Met Phe Ile Pro Gln Ile Ala Val Gln Ile Tyr Trp Val Leu Asn
 210 215 220
 Lys Thr Met Leu Gly Ser Leu Asp Ser Val Thr Ser Ser Gly Phe Phe
 225 230 235 240
 Asp Gln Ser Asp Lys Ile Val Lys Leu Val Leu Ala Ile Ala Thr Ala
 245 250 255
 Thr Gly Thr Val Met Leu Pro Arg Val Ala Asn Ala Phe Ala His Arg
 260 265 270
 Glu Tyr Ser Lys Ile Lys Glu Tyr Met Tyr Ala Gly Phe Ser Phe Val
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 Ser Ala Ile Ser Ile Pro Met Met Phe Gly Leu Ile Ala Ile Thr Pro
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 Lys Phe Val Pro Leu Phe Phe Thr Ser Gln Phe Ser Asp Val Ile Pro
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 Val Leu Met Ile Glu Ser Ile Ala Ile Ile Phe Ile Ala Trp Ser Asn
 325 330 335
 Ala Ile Gly Thr Gln Tyr Leu Leu Pro Thr Asn Gln Asn Lys Ser Tyr
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 Thr Val Ser Val Ile Ile Gly Ala Ile Val Asn Leu Met Leu Asn Ile
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 Pro Leu Ile Ile Tyr Leu Gly Thr Val Gly Ala Ser Ile Ala Thr Val
 370 375 380
 Ile Ser Glu Met Ser Val Thr Val Tyr Gln Leu Phe Ile Ile His Lys
 385 390 395 400
 Gln Leu Asn Leu His Thr Leu Phe Ala Asp Leu Ser Lys Tyr Leu Ile
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 Ala Gly Leu Val Met Phe Leu Ile Val Phe Lys Ile Ser Leu Leu Thr
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 Pro Thr Ser Trp Ile Phe Ile Leu Leu Glu Ile Thr Val Gly Ile Ile
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 Ile Tyr Val Val Leu Leu Ile Phe Leu Lys Ala Glu Ile Ile Asn Lys
 450 455 460
 Leu Lys Phe Ile Met His Lys
 465 470